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(54) Title: HUMAN KCNQ5 POTASSIUM CHANNEL, METHODS AND COMPOSITIONS THEREOF

(57) Abstract: An isolated polynucleotide encoding a novel potassium channel polypeptide, KCNQ5, that is expressed primarily in brain and skeletal muscle is described. The new polypeptide has been cloned and isolated from a human brain cDNA library and is a member the KCNQ family of potassium channels. The provided human KCNQ5 nucleic acid sequence and encoded polypeptide can be employed for diagnostic, screening and therapeutic uses. Moreover, the hKCNQ5 polypeptide can be used to assay for KCNQ5 potassium channel modulators, which can be utilized in the treatment of neurological, neurophysiological, neuropsychological and neuroaffective diseases, conditions and disorders, including, but not limited to, acute and chronic pain, migraine, acute stroke, dementia, vascular dementia, trauma, epilepsy, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Parkinson's Disease, learning and cognitive disorders, and neurophysiological disorders including anxiety disorders, depression, bipolar disorders, sleep disorders, addiction, and eating disorders.

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HUMAN KCNQ5 POTASSIUM CHANNEL, METHODS AND COMPOSITIONS THEREOF

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[0001] This application claims benefit of provisional patent application U.S. Serial No. 60/207,389, filed May 26, 2000.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the KCNQ family of
10 potassium channels. More specifically, the present invention provides a new potassium channel polypeptide, KCNQ5, which was cloned and isolated from human tissue. Human KCNQ5 is found to be expressed primarily in brain and skeletal muscle. The present invention further provides methods for modulating the KCNQ5 potassium channel and assays for detecting
15 channel modulators, which have use in the treatment of a variety of neurological, neurophysiological and neuropsychological conditions, disorders and diseases.

BACKGROUND OF THE INVENTION

[0003] Potassium channels are membrane-spanning proteins that
20 generally act to hyperpolarize neurons. Physiological studies indicate that potassium currents are found in most cells and are associated with a wide range of functions, including the regulation of the electrical properties of excitable cells. Depending on the type of potassium channel, its functional activity can be controlled by transmembrane voltage, different ligands,
25 protein phosphorylation, or other second messengers.

[0004] In the last decade, the cloning of potassium channels has resulted in the discovery of the molecular isolation and characterization of greater than 50 potassium channel genes and many of their associated regulatory subunits. More recently, a new family of potassium channel genes, the
30 KCNQ gene family, has been described (N.A. Singh et al., 1998, Nature

Genet., 18:25-29; C. Charlier et al., 1998, Nature Genet., 18:53-55; C. Biervert et al., 1998, Science, 279:403-406). The KCNQ family of potassium channels are voltage dependent potassium channels. They contain the voltage sensor and pore signature sequences characteristic of a 6

5 transmembrane potassium channel gene and have a longer carboxy-terminus than other known voltage-dependent potassium channels.

[0005] A remarkable aspect about the KCNQ1-4 gene family is that mutations in each channel are associated with a particular disease, including cardiac arrhythmias (KCNQ1), epilepsy (KCNQ2 and KCNQ3) , and hearing

10 loss (KCNQ4). The present invention provides a newly isolated, cloned and characterized member of the KCNQ family, called KCNQ5. Human KCNQ5 provides the art with an additional member of the KCNQ family of potassium channel proteins, isolated from a human source, for use in the methods and compositions described herein.

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SUMMARY OF THE INVENTION

[0006] It is an object of the present invention to provide a novel isolated polynucleotide molecule encoding a novel potassium channel polypeptide referred to herein as KCNQ5, or hKCNQ5 (i.e., human KCNQ5). The present invention encompasses the amino acid sequence of the hKCNQ5

20 protein and the nucleic acid sequence encoding the hKCNQ5 protein. Also embraced by the present invention are variations in the hKCNQ5 nucleic acid sequence due to degeneracy in the genetic code.

[0007] The present invention provides for polynucleotide molecules which are at least about 70% identical to the polynucleotide sequence of the native

25 hKCNQ5 sequence disclosed herein and set forth as SEQ ID NO:1.

Preferably, the present invention provides: (a) a purified and isolated nucleic acid molecule encoding a KCNQ5 protein according to the present invention; (b) nucleic acid sequences complementary to (a); (c) nucleic acid sequences having at least 70% sequence identity, preferably at least 80% to at least

30 90%, more preferably at least 95%, and most preferably at least 98%

sequence identity to (a); or (d) a fragment of (a) or (b) which will hybridize to (a) or (b) under low, moderately stringent and/or highly stringent conditions (described further herein), said fragment preferably comprising at least 15 nucleotides to 20 nucleotides (e.g., encoding at least 5 codons, more preferably at least about 7-15 codons), and more preferably encoding a functional or biologically active fragment of the hKCNQ5 polypeptide, such as the pore region (which spans about amino acid 191 to amino acid 209 of the KCNQ5 polypeptide sequence) or the S4 voltage sensor region (which spans about amino acid 265 to amino acid 285 of the KCNQ5 polypeptide sequence) (Fig. 5A).

[0008] It is another object of the present invention to provide an amino acid sequence which is at least about 70% to 80% identical to the hKCNQ5 polypeptide sequence disclosed herein and set forth as SEQ ID NO:2. Preferably, the present invention includes: (a) the amino acid sequence of the KCNQ5 protein of the present invention; and (b) amino acid sequences having at least 70% sequence identity, preferably at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 98% sequence identity to (a).

[0009] It is yet a further object of the present invention to provide a novel KCNQ5 polynucleotide molecule, associated vectors, host cells, and methods of use. Preferably, the polynucleotide molecule is a DNA molecule. In accordance with the present invention, polynucleotide sequences having approximately 70% or greater identity to the hKCNQ5 sequence are preferred, preferably under moderate or high stringency conditions. Also preferred are nucleotide sequences that about 80% or more identical to the KCNQ5 polynucleotide sequence of SEQ ID NO:1.

[0010] Yet another object of the present invention is to provide nucleic acids obtained by PCR with degenerate oligonucleotide primers. The ordinarily skilled practitioner in the art can devise such primers based on the hKCNQ5 nucleic acid sequence and/or the consensus sequence(s) described herein using techniques known and practiced in the art. For

example, PCR techniques are described in White et al., 1989, Trends Genet. 5: 185-9.

- [0011]** Another object of the present invention is to provide expression vectors comprising a nucleic acid sequence coding for a KCNQ5 polypeptide, or fragment thereof, preferably a functional or biologically active fragment thereof; host cells containing such vectors; and polypeptides comprising the amino acid sequence of the KCNQ5 protein. Such polypeptides, or fragments thereof, may be isolated and purified employing conventional methodologies, following expression in the host cell.
- 5
- 10 Preferably, the vector encodes a full-length KCNQ5 protein and the polypeptide is a full-length KCNQ5 protein. Preferred are frog (*Xenopus*) expression vectors, such as pSP64T or derivatives thereof (Melton et al., 1984, Nucl. Acids Res., 12: 7057-70); mammalian cell expression vectors, such as pcDNA3 (available from Invitrogen); or bacterial cell expression
- 15 vectors, such as pET-30 (available from Novagen or Promega).

- [0012]** Yet another object of the present invention is to provide host cells transformed with the above-described vectors. Preferred are *Xenopus* oocytes, mammalian cells (e.g., HEK-293, CHO, L929), and bacterial cells (e.g., *E. coli*, especially BL21(DE3), available from Novagen). Particularly
- 20 preferred are HEK-293 cells deposited as ATCC Accession No. CRL-1573 (American Type Culture Collection, 10801 University Boulevard, Manassas VA 20110-2209). The present invention also provides a method for producing a KCNQ5 polypeptide having the amino acid sequence as depicted in SEQ ID NO:2 by culturing a host cell under conditions suitable
- 25 for the expression of the polypeptide, and recovering the polypeptide from the host cell culture.

- [0013]** It is another object of the present invention to provide methods for detecting nucleic acids that code for KCNQ5 proteins, as well as processes for detecting molecules that bind to and/or otherwise modulate (e.g.,
- 30 activate, up-regulate, increase, or inhibit, block, or down-regulate) the activity of the KCNQ5 protein. As used herein, "modulate" encompasses

both channel openers/activators, and the like, as well as channel closers/inactivators/blockers, and the like.

[0014] A further object of the present invention is to provide methods of modulating KCNQ5 proteins, specifically methods of opening/activating or closing/inactivating/blocking KCNQ5 potassium channels. Moreover, the present invention encompasses a method of treating a disease or disorder, preferably, a neuroaffective disorder, such as a neurological, neurophysiological, or neuropsychological disease or disorder, by modulating the activity of the KCNQ5 protein. Thus, the present invention is directed to a treatment of an individual in need of such treatment for a condition that is mediated by a potassium channel, particularly, the KCNQ5 potassium channel, or for a condition that is mediated by the biological activity of human KCNQ5, comprising administering to the individual a potassium channel modulating compound in an amount effective to modulate the activity of the potassium channel as described herein.

[0015] Another object of the present invention is to provide methods for identifying compounds (e.g., small molecules, peptides, analogs, mimetics) that modulate the biological activity of a potassium channel, preferably the hKCNQ5 potassium channel, comprising: combining a candidate compound modulator of a potassium channel biological activity with the KCNQ5 potassium channel polypeptide having the amino acid sequence set forth in SEQ ID NO:2; and measuring an effect of the candidate compound modulator on the biological activity of the potassium channel polypeptide. According to the present invention, the potassium channel polypeptide may be expressed by a recombinant host cell.

[0016] It is a further object of the present invention to provide an antisense polynucleotide molecule comprising substantially the complement of the KCNQ5 nucleic acid sequence set forth in SEQ ID NO:1, or a biologically effective portion thereof. Also, in accordance with the present invention is provided a method for modulating, e.g., inhibiting or downregulating, the expression and/or activity of the KCNQ5 potassium channel polypeptide

using the antisense polynucleotide molecule, comprising administering to an individual in need of such modulating an amount of the antisense molecule effect to modulate the expression and/or activity of the KCNQ5 potassium channel polypeptide.

- 5 **[0017]** Yet a further object of the present invention is to provide an antibody having specificity toward an isolated and purified KCNQ5 polypeptide, preferably the hKCNQ5 polypeptide, having the amino acid sequence as shown in SEQ ID NO:2, or an immunoreactive peptide fragment thereof.
- 10 **[0018]** Additional objects and advantages afforded by the present invention will be apparent from the detailed description hereinbelow.

DESCRIPTION OF THE FIGURES

- 15 **[0019]** The appended drawings of the figures are presented to further describe the invention and to assist in its understanding through clarification of its various aspects.

[0020] Figures 1A-1D show the coding sequence (i.e., cDNA) of human KCNQ5 from nucleotides 1 to 2694. (SEQ ID NO:1).

- 20 **[0021]** Figures 2A and 2B show the amino acid sequence (SEQ ID NO:2) of the human KCNQ5 polypeptide deduced from the coding sequence shown in Figures 1A-1D.

[0022] Figure 3 shows an alternative splice exon of hKCNQ5, which is located between nucleotide positions 1143 and 1144 (SEQ ID NO:3) in the hKCNQ5 coding sequence.

- 25 **[0023]** Figures 4A and 4B show the 3' untranslated region (3' UTR), (SEQ ID NO:4) of the hKCNQ5 sequence.

- 30 **[0024]** Figures 5A-5C show multiple sequence alignments of the human KCNQ channel family members. The blackened areas represent identical amino acids among all 5 family members and the gray highlighted amino acids represent similar amino acids among the KCNQ family members. As shown in Figures 5A-5C: human KCNQ1 (SEQ ID NO:28); human KCNQ2

(SEQ ID NO:29); human KCNQ3 (SEQ ID NO:30); human KCNQ4 (SEQ ID NO:31); human KCNQ5 (Figures 2A/2B and SEQ ID NO:2).

[0025] Figures 6A and 6B show mRNA localization of hKCNQ5 by dot blot analysis. Human KCNQ5 is observed in whole brain and brain subregions, including caudate nucleus, cerebellum, hippocampus, cerebral cortex, frontal lobe, occipital pole, putamen and temporal lobe, as well in skeletal muscle. Figure 6A presents Table 1, a Human RNA Master Blot, showing type and position of poly A⁺ RNAs and controls that are spotted or dotted on the membrane. Figure 6B shows the actual dot blot wherein a high level of hybridization of hKCNQ5 probe has occurred specifically in spots on the membrane occupied by mRNA samples from human tissues as follows: 1A: whole brain; 3A: caudate nucleus; 5A: cerebral cortex; 6A: frontal lobe; 7A: hippocampus; 1B: occipital lobe; 2B: putamen; 4B: temporal lobe; 3C: skeletal muscle; and 1G: fetal brain (slight).

[0026] Figure 7 shows a Northern blot analysis in which hKCNQ5 mRNA is found to be localized to skeletal muscle and brain. A single transcript of approximately 7.5 Kb is shown. The lanes contain mRNA from the following tissues, left to right: brain, heart, skeletal muscle, colon (no mucosa), thymus, spleen, kidney, liver, small intestine, placenta, lung and peripheral blood leukocytes (PBL). Molecular weight markers (kd) are shown in the leftmost lane of the blot: 9.5, 7.5, 4.4, 2.4, 1.35 and 0.24.

[0027] Figure 8 shows that expression of hKCNQ5 in *Xenopus* oocytes results in large non-activating outward currents that are resistant to blockage by triethylammonium (TEA) at high concentrations. (Example 3).

[0028] Figures 9A-9E show the results of *in situ* hybridization studies using KCNQ5 antisense and sense probes (Example 3) on brain sections. Specifically shown are autoradiograms of representative coronal sections of rat brain showing positive *in situ* hybridization signal with antisense (Figs. 9A-9C) or sense (control), (Figs. 9D-9F) riboprobes for human KCNQ5 mRNA. The abbreviations in Fig. 9A are as follows: Ctx: cortex; CPu: caudate putamen ; Pir: piriform cortex; SHipp: septohippocampal nucleus.

The abbreviations in Figure 9B are as follows: CA2 and CA3 are regions of the hippocampus. The abbreviations in Fig. 9C are as follows: Pn: pontine nuclei; RtTg: reticulotegmental nuclei of the pons.

5 **[0029]** Figures 10A and 10B depict dark field images of emulsion dipped slides showing the expression of KCNQ5 mRNA in the hippocampus. Fig. 10A: Hybridization with the antisense riboprobe shows positive signal in the CA3 region, which is absent in the dentate gyrus (DG). Fig. 10B: The signal is absent with the sense riboprobe.

10 **[0030]** Figure 11 depicts dark field image of emulsion dipped slides showing that the expression of KCNQ5 mRNA is present in the CA2 region, but absent in the CA1 region of the hippocampus.

15 **[0031]** Figures 12A and 12B depict dark field images of emulsion dipped slides showing the expression of KCNQ5 mRNA in the cortex. Hybridization with the antisense riboprobe shows a weak, diffuse positive signal with clusters over individual neurons (Fig. 12A), which is absent with the sense riboprobe (Fig. 12B).

20 **[0032]** Figures 13A and 13B depict dark field images of emulsion dipped slides showing the expression of KCNQ5 mRNA in the piriform cortex. Hybridization with the antisense riboprobe shows a weak-moderate signal (Fig. 13A), which is absent with the sense riboprobe (Fig. 13B).

25 **[0033]** Figures 14A and 14B depict dark field images of emulsion dipped slides showing the expression of KCNQ5 mRNA in the septohippocampal nucleus. Hybridization with the antisense riboprobe shows a weak signal (Fig. 14A), which is absent with the sense riboprobe (Fig. 14B).

30 **[0034]** Figures 15A and 15B depict dark field images of emulsion dipped slides showing the expression of KCNQ5 mRNA in the reticulotegmental nucleus of the pons. Hybridization with the antisense riboprobe shows a weak-moderate signal, with clusters of grains over individual neurons (Fig. 15A), which is absent with the sense riboprobe (Fig. 15B).

30 **[0035]** Figures 16A and 16B depict dark field images of emulsion dipped slides showing the expression of KCNQ5 mRNA in the pontine nuclei.

Hybridization with the antisense riboprobe shows a weak signal (Fig. 16A), which is absent with the sense riboprobe (Fig. 16B).

[0036] Figures 17A and 17B depict bright field (Fig. 17A) and dark field (Fig. 17B) images of emulsion dipped slides showing the expression of
5 KCNQ5 mRNA in the dorsal root ganglion (DRG). Hybridization with the antisense riboprobe shows a weak signal in about 20-30% of the neurons (large white arrows), (Fig. 17B) which is absent in other neurons (smaller black arrows), (Fig. 17B).

[0037] Figures 18A and 18B depict bright field (Fig. 18A) and dark field
10 (Fig. 18B) images of emulsion dipped slides showing the expression of KCNQ5 mRNA in the trigeminal ganglion. Hybridization with the antisense riboprobe shows a weak signal in about 20-30% of the neurons (large white arrows), (Fig. 18B), which is absent in other neurons (smaller, white-outlined arrows).

15 DETAILED DESCRIPTION OF THE INVENTION

[0038] The present invention provides an isolated and purified polynucleotide encoding a new human potassium channel polypeptide, called human KCNQ5 (hKCNQ5). The invention relates to the isolation, cloning and sequencing the hKCNQ5 potassium channel gene from human
20 tissue. A newly discovered member of the KCNQ family of potassium channels, hKCNQ5 has a limited tissue distribution and can be used in the identification and characterization of modulators of this potassium channel. Such modulators can affect channel activity/function, particularly with respect to neuroaffective diseases, or neurological, neurophysiological, or
25 neuropsychological diseases, conditions and disorders, preferably those affecting or influencing brain and/or skeletal muscle.

Definitions

[0039] Unless otherwise defined, the technical and scientific terms as used herein have the same meanings as are commonly understood by
30 persons skilled in the art to which the present invention pertains. The

following definitions apply to the terms used throughout this specification, unless otherwise defined in specific instances:

[0040] Biological activity or functional activity refers to the ability to allow transmembrane potassium ion flow and/or transport, or to regulate
5 transmembrane potassium ion flow and/or transport, or to the ability of a subunit to bind to another subunit, ligand, or cofactor, and/or to otherwise modulate the pharmacological activity of a potassium channel.

[0041] Cloning refers to the isolation of a particular gene from genetic material, for example a genome, genomic library, or cDNA library into a
10 plasmid or other vector.

[0042] Purified refers to molecules, either polynucleotides or amino acids (polypeptides and proteins) that are removed from their natural environment and isolated or separated from at least one other component with which they are naturally associated. Polynucleotides include nucleic acids, namely,
15 DNA, cDNA, genomic DNA, RNA, synthetic forms and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified, or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those skilled in the art.

[0043] In general, a polypeptide refers to a polymer of amino acids and its
20 equivalent, and does not refer to a specific length of the product. Peptides, oligopeptides and proteins may be termed polypeptides. The terms polypeptide and protein are often used interchangeably herein. The term polypeptide also does not refer to, or exclude, modifications of the polypeptide, e.g., glycosylation, acetylation, phosphorylation and the like.
25 Included in the definition of KCNQ5 polypeptides are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, and the like), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally and non-naturally occurring.

[0044] The term hybridization refers to any process by which a strand of
30 nucleic acid binds with a complementary strand through base pairing. A

hybridization complex refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary G and C bases and between complementary A and T bases; these hydrogen bonds may be further stabilized by base stacking interactions. The two complementary nucleic acid sequences hydrogen bond in an antiparallel configuration. A hybridization complex may be formed in solution (e.g., C₀t or R₀t analysis) or between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., membranes, filters, chips, pins or glass slides to which cells have been fixed *in situ* hybridization).

[0045] The terms complementary or complementarity refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A". Complementarity between two single-stranded molecules may be "partial", in which only some of the nucleic acids bind, or it may be complete when total complementarity exists between single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands.

[0046] Homology refers to a degree of complementarity. There may be partial homology, or complete homology, which is equivalent to identity. A partially complementary sequence is one that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid; it can be referred to using the functional term "substantially homologous". The inhibition of hybridization of a completely complementary sequence to a target sequence can be examined using a hybridization assay (e.g., Southern or Northern blot, solution hybridization, and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (i.e., the hybridization) of a

completely homologous sequence or probe to the target sequence under conditions of low stringency.

[0047] The terms "identical" or "percent identity" in the context of two or more nucleic acid or amino acid sequences, refer to two or more sequences
5 or subsequences that are the same, or that have a specified percentage of amino acid residues or nucleotides that are the same (e.g., 60% or 65% identity, preferably, 70%-95% identity, more preferably, >95% identity), when compared and aligned for maximum correspondence over a window of comparison, or over a designated region as measured using a sequence
10 comparison algorithm as known in the art, or by manual alignment and visual inspection. Sequences having, for example, 60% to 95% or greater sequence identity are considered to be substantially identical. Such a definition also applies to the complement of a test sequence. Preferably the described identity exists over a region that is at least about 15 to 25 amino
15 acids or nucleotides in length, more preferably, over a region that is about 50 to 100 amino acids or nucleotides in length.

[0048] Those having skill in the art will know how to determine percent identity between/among sequences using, for example, algorithms such as those based on the CLUSTALW computer program (J.D. Thompson et al.,
20 1994, *Nucleic Acids Research*, 2(22):4673-4680), or FASTDB, (Brutlag et al., 1990, *Comp. App. Biosci.*, 6:237-245), as known in the art. Although the FASTDB algorithm typically does not consider internal non-matching deletions or additions in sequences, i.e., gaps, in its calculation, this can be corrected manually to avoid an overestimation of the % identity.

25 CLUSTALW, however, does take sequence gaps into account in its identity calculations. Also available to those having skill in this art are the BLAST and BLAST 2.0 algorithms (Altschul et al., 1977, *Nuc. Acids Res.*, 25:3389-3402 and Altschul et al., 1990, *J. Mol. Biol.*, 215:403-410). The BLASTN program for nucleic acid sequences uses as defaults a wordlength (W) of
30 11, an expectation (E) of 10, M=5, N=4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a

wordlength (W) of 3, and an expectation (E) of 10. The BLOSUM62 scoring matrix (Henikoff & Henikoff, 1989, *Proc. Natl. Acad. Sci., USA*, 89:10915) uses alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

- 5 **[0049]** As known in the art, numerous equivalent conditions may be employed to comprise either low or high stringency conditions. Factors such as the length and nature (DNA, RNA, base composition – G + C content) of the sequence, nature of the target (DNA, RNA, base composition, presence in solution or immobilization, etc.), and the concentration of the salts and
- 10 other components (e.g., the presence or absence of formamide, dextran sulfate and/or polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions of either low or high stringency different from, but equivalent to, the above listed conditions.
- [0050]** The phrase stringency of hybridization refers to conditions under
- 15 which polynucleic acid hybrid molecules are stable. As known to those skilled in the art, the stability of a hybrid is reflected in the melting temperature (T_m) of the hybrids. Also as is appreciated by the skilled practitioner, T_m can be approximated by the formulas as known in the art, depending on a number of parameters, such as the length of the hybrid or
- 20 probe in number of nucleotides, or hybridization buffer ingredients and conditions (See, for example, T. Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982 and J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989; Current Protocols
- 25 in Molecular Biology, Eds. F.M. Ausubel et al., Vol. 1, "Preparation and Analysis of DNA", John Wiley and Sons, Inc., 1994-1995, Suppls. 26, 29, 35 and 42; pp. 2.10.7- 2.10.16; G.M. Wahl and S. L. Berger (1987; Methods Enzymol. 152:399-407); and A.R. Kimmel, 1987; Methods of Enzymol. 152:507-511). As a general guide, T_m decreases approximately 1°C –1.5°C
- 30 with every 1% decrease in sequence homology. Also, in general, the stability of a hybrid is a function of sodium ion concentration and

temperature. Typically, the hybridization reaction is initially performed under conditions of low stringency, followed by washes of varying, but higher stringency. Reference to hybridization stringency, e.g., high, moderate, or low stringency, generally relates to such washing conditions.

- 5 **[0051]** Thus, for example, high stringency refers to conditions that permit hybridization of those nucleic acid sequences that form stable hybrids in 0.018M NaCl at 65°C (i.e., if a hybrid is not stable in 0.018M NaCl at 65°C, it will not be stable under high stringency conditions). High stringency conditions can be provided, for instance, by hybridization in 50% formamide,
- 10 5x Denhart's solution, 5xSSPE (1x SSPE buffer comprises 0.15 M NaCl, 10 mM Na₂HPO₄, 1 mM EDTA), (or 1x SSC buffer containing 150 mM NaCl, 15 mM Na₃ citrate • 2 H₂O, pH 7.0), 0.2% SDS at 42°C, followed by washing in 1x SSPE (or SSC) and 0.1% SDS at a temperature of at least about 42°C, preferably about 55°C, more preferably about 65°C.
- 15 **[0052]** Moderate stringency refers, for example, to conditions that permit hybridization in 50% formamide, 5x Denhart's solution, 5xSSPE (or SSC), 0.2% SDS at 42°C, followed by washing in 0.2x SSPE (or SSC) and 0.2% SDS at a temperature of at least about 42°C, preferably about 55°C, more preferably about 65°C.
- 20 **[0053]** Low stringency refers, for example, to conditions that permit hybridization in 10% formamide, 5x Denhart's solution, 6xSSPE (or SSC), 0.2% SDS at 42°C, followed by washing in 1x SSPE (or SSC) and 0.2% SDS at a temperature of about 45°C, preferably about 50°C.
- 25 **[0054]** For additional stringency conditions, see T. Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982). It is to be understood that the low, moderate and high stringency hybridization / washing conditions may be varied using a variety of ingredients, buffers and temperatures well known to and practiced by the skilled practitioner.
- 30 **[0055]** Multi-copy plasmid refers to a plasmid having greater than one copy present in a cell (typically 10 to 30 copies).

[0056] Northern blotting refers to a method of identifying particular RNA fragments, particularly mRNA, by hybridization with a complementary nucleic acid, typically a cDNA or an oligonucleotide. Oligonucleotides or oligomers refer to a nucleic acid sequence, preferably comprising
5 contiguous nucleotides, of at least about 6 nucleotides to about 60 nucleotides, preferably at least about 8 to 10 nucleotides in length, more preferably at least about 12 nucleotides in length e.g., about 15 to 35 nucleotides, or about 15 to 25 nucleotides, or about 20 to 35 nucleotides, which can be typically used in PCR amplification assays, hybridization
10 assays, or in microarrays. It will be understood that the term oligonucleotide is substantially equivalent to the terms primer, probe, or amplimer, as commonly defined in the art. It will also be appreciated by those skilled in the pertinent art that a longer oligonucleotide probe, or mixtures of probes, e.g., degenerate probes, can be used to detect longer, or more complex,
15 nucleic acid sequences, for example, genomic DNA. In such cases, the probe may comprise at least 20-200 nucleotides, preferably, at least 30-100 nucleotides, more preferably, 50-100 nucleotides.

[0057] Open reading frame, or "ORF", refers to a DNA sequence containing a series of nucleotide triplets coding for amino acids and typically
20 lacking any termination codons.

[0058] Plasmid refers to cytoplasmic, autonomously replicating DNA elements, which are either exogenously added to, or endogenously present in, microorganisms.

[0059] Promoter refers to a region on DNA at which RNA polymerase
25 binds and initiates transcription.

[0060] Southern blotting refers to a method of identifying particular DNA fragments by hybridization with a complementary nucleic acid, typically a cDNA or an oligonucleotide.

[0061] Modulation refers to the capacity to either enhance, augment,
30 activate, or decrease, diminish, or inhibit a functional, biophysical, or biological activity of the KCNQ5 potassium ion channel. Modulation or

regulation of biological activity refers to binding, blocking, inhibiting, antagonizing, repressing, neutralizing, or sequestering, either completely or partially, a KCNQ5 potassium channel protein, including, but not limited to, the human KCNQ5 channel described herein, as well as up-regulating, agonizing, increasing, or activating the KCNQ5 potassium channel by a compound that can be identified by the methods described herein. Inhibitors are compounds that inhibit, decrease, block, prevent, delay activation, inactivate, desensitize, or down-regulate the ion channel, or speed up or enhance deactivation. Activators are compounds that increase, open, activate, facilitate, enhance activation, sensitize, or up-regulate channel activity, or delay or slow inactivation.

[0062] Assays for inhibitors and activators include, for example, expressing a recombinantly produced KCNQ5 potassium channel in cells or cell membranes and then measuring the flux of potassium ions through the channel, either directly or indirectly. Alternatively, cells expressing endogenous KCNQ5 channels can be used in such assays. To examine the extent of inhibition, samples or assays comprising a KCNQ5 potassium channel are treated with a potential activator or inhibitor compound and are compared with control samples without the test compound. For example, control samples (untreated with test compounds) are assigned a relative potassium ion channel activity value of 100%. Inhibition of a KCNQ5 potassium ion channel is achieved when the KCNQ5 channel activity value relative to the control is about 90%, preferably about 50%, more preferably about 25 to 0%. Activation of an ion channel is achieved when the ion channel activity value relative to the control is greater than 100% to 110%, preferably, 150%, more preferably, at least 200-500% or higher, most preferably, at least 1000%, or higher.

[0063] A biologically active fragment refers to portions of the KCNQ5 potassium channel polypeptide, such as peptides, that have been truncated with respect to the N- or C- termini, or both; or to the corresponding 5' or 3' end, or both, of the corresponding polynucleotide coding region. The

portions or fragments perform essentially the same biological function, or encode peptides which perform essentially the same function in substantially the same way as the KCNQ5 polypeptide. Biologically active can also refer to the activity of a homologue, variant, or analog entity that has structural, regulatory, or biochemical functions that are essentially the same as the naturally occurring, or recombinantly-expressed, entire KCNQ5 polypeptide entity.

[0064] For definitions of other terms in this specification, F. Sherman et al., Laboratory Course Manual for Methods in Yeast Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1987) and Lewin, B., Genes IV, Oxford University Press, Oxford (1990), serve as guides.

[0065] In one aspect of the present invention, the novel hKCNQ5 protein, which is highly likely to be involved in neurotransmission and neurophysiological functions, serves as a target for identifying modulators of the potassium channel. KCNQ5 modulators would be useful as diagnostic agents, therapeutic agents and/or in the treatment of central nervous system-associated diseases, neurological diseases, conditions and physiological disorders associated with or linked to the opening or blocking of the KCNQ5 potassium channel, including, but not limited to, acute and chronic pain, migraine headaches, acute stroke, cognitive disorders, dementia, vascular dementia, trauma, epilepsy, seizures, affective disorders, amyelotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Parkinson's Disease, Parkinson's-like motor disorders, and neurophysiological, or neuropsychological disorders including anxiety disorders, phobias, depression, Pick's disease, psychosis, bipolar disorders, sleep disorders, compulsive behavior, addiction, and eating disorders, ataxia, myokymia, Alzheimer's disease, age-associated memory loss and other cognitive disorders, learning deficiencies, motor neuron diseases, spinal cord damage, tremor seizures, convulsions, and the like. Openers of the hKCNQ5 potassium channel are of particular interest for increasing the flow of ions across a cell membrane and affecting one or more of the above-

listed conditions or diseases, for example, migraine, pain, or anxiety disorders.

[0066] The novel KCNQ5 potassium channel protein described herein can play a crucial role in modulating synaptic transmission and electrical excitability in the brain. Modulating the function of the KCNQ5 protein of the present invention may have significant implications in cognitive disorders (e.g., learning and memory), behavioral disorders (e.g., anxiety and/or depression), psychiatric conditions (e.g., bipolar disorders, schizophrenia), neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease), developmental disorders (e.g., mental retardation), as well as in other physiological and/or neurophysiological or neuropsychological problems or disorders, such as asthma, migraine headaches, seizures, i.e., epileptic seizures, stroke, neuronal cell death, chronic and acute pain, and brain tumors.

[0067] In another embodiment of the invention, the KCNQ5 nucleic acid provided herein, or antisense nucleic acids complementary to the KCNQ5 nucleic acid sequence, or portions thereof, may be utilized as therapeutic or diagnostic agents. For therapy involving KCNQ5 polynucleotide molecules, the nucleic acids may be incorporated into vectors and/or formulated as described hereinbelow and as known and practiced in the art.

[0068] In addition, persons skilled in the art can use the KCNQ5 polypeptides and nucleic acids of this invention to prepare antibodies, vectors, cells and/or and cell lines. All of these are useful in assays for the identification of KCNQ5 protein modulators, as well as in therapeutic, screening and diagnostic applications.

[0069] Further, KCNQ5 protein modulators can be administered to various mammalian species, such as monkeys, dogs, cats, mice, rats, humans, etc. By known methods, persons skilled in the pharmaceutical art can incorporate KCNQ5 protein modulators in a conventional systemic dosage form, such as a tablet, capsule, elixir or injectable formulation. The above dosage forms will also include any necessary physiologically acceptable

carrier material, excipient, lubricant, buffer, antibacterial, bulking agent (such as mannitol), anti-oxidants (ascorbic acid or sodium bisulfite) or the like, as further described *infra*.

Cloning and Isolation of KCNQ5 Nucleic Acid

5 **[0070]** In one aspect of the present invention, the cloning, sequencing and functional expression of a full-length human cDNA clone of hKCNQ5, preferably the hKCNQ5 nucleic acid sequence set forth in SEQ ID NO:1 and Figures 1A-1D are provided. A DNA clone comprising the full-length human KCNQ5 cDNA (in pcDNA3.1) of the present invention was deposited with
10 the American Type Culture Collection (ATCC) (10801 University Blvd., Manassas, VA 20110-2209) on May 26, 2000, ATCC Accession Number PTA-1924. The deposit(s) referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for purposes of Patent Procedure. These deposits are
15 provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. 112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any
20 conflict with any description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

[0071] KCNQ5 nucleic acid can be obtained by known methods through the use of the described human KCNQ5 polynucleotide sequence of the
25 present invention. For example, methods such as Southern and Northern blotting, Western immunoblotting, chemical synthesis, synthesis by polymerase chain reaction (PCR) from primers obtainable from SEQ ID NO:1, Figures 1A-1D and as described herein, expression cloning; and subtractive cDNA cloning are all available to the skilled practitioner for use in
30 obtaining KCNQ5 derivable from other animal sources, including human and non-human mammals.

[0072] The nucleic acids encoding the hKCNQ5 protein of the present invention can be modified to prepare useful mutations. For example, one may modify the sequence to provide additional restriction endonuclease recognition sites in the nucleic acid. Such mutations may be silent or may
5 change the amino acid encoded by the mutated codon. Moreover, modified nucleic acids can be prepared, for example, by mutating the nucleic acid coding for hKCNQ5 to result in deletion, substitution, insertion, inversion or addition of one or more amino acids in the encoded polypeptide. For methods of site-directed mutagenesis, see Taylor, J. W. et al., 1985, Nucl.
10 Acids Res., 13, 8749-64 and Kunkel, J. A., 1985, Proc. Natl. Acad. Sci. U.S.A. 82: 482-92. In addition, kits for site-directed mutagenesis are available from commercial vendors (e.g., BioRad Laboratories, Richmond, CA; Amersham Corp., Arlington Heights, IL). For disruption, deletion and truncation methods, see Sayers, J. R. et al., 1988, Nucl. Acids Res., 16:
15 791-800.

[0073] The present invention also embraces modified nucleic acids, including (1) alternative splice exon variants, e.g., SEQ ID NO:3; (2) allelic variants; and (3) chimeric potassium channels in which the fusion construct comprises a KCNQ5 modulatory site. Such modified nucleic acids can be
20 obtained by persons of ordinary skill in the art using the sequences provided herein. Accordingly, the KCNQ5 nucleotide sequence of the present invention may be molecularly engineered to alter the coding sequence, so as to advantageously modify the cloning, processing and/or expression of the gene product. For example, mutations may be introduced into the
25 sequence using techniques that are well-known in the art and as described *supra*, e.g., site-directed mutagenesis to insert one or more new restriction sites, to alter glycosylation patterns, or to change codon preference or usage, to mention a few.

[0074] Also contemplated within the scope of the present invention are
30 alleles of the human KCNQ5 protein described herein. As understood by those in the art, an allele or allelic sequence is an alternative form of the

potassium channel described herein. Alleles result from nucleic acid mutations and mRNA splice-variants which produce polypeptides whose structure or function may or may not be altered. Any given gene may have none, one, or many, allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions, or substitutions of amino acids. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given nucleic acid sequence.

[0075] In a particular aspect, the cloning of hKCNQ5 of the present invention involved the isolation of molecular clones as specifically described in Example 1 herein, which were sequenced and analyzed by searching the NCBI database (i.e., GenBank) for sequence homologies at a level of between about 35-45%. The results showed greater than about 40% homology with the known KCNQ genes within the databases. In addition, several of the isolated clones were aligned with each other in order to determine start and stop sequence codons. Although none of the individual clones appeared to comprise full length sequence, they did have substantial open reading frames. Several of the isolated clones were found to contain the 5' ATG start codon and others contained the 3' TAA stop codon. The alignment of all of the clone sequences generated a full length open reading frame of 2694 nucleotides. The full length DNA coding region of hKCNQ5 according to this invention is shown in Figures 1A-1D and is set forth in SEQ ID NO:1.

[0076] Hydropathy analysis indicated the presence of 6 membrane spanning domains and one pore region. The hKCNQ5 amino acid sequence (SEQ ID NO:2) revealed a KCNQ5 protein having two distinguishing features that are characteristics of potassium channel genes; namely, 1) the signature 'Gly-Tyr-Gly' pore sequence, and 2) alternating charged residues (Arg--Arg--Glu--Arg) in the S4 membrane-spanning domain, indicative of a voltage sensor.

[0077] One of the isolated clones contained an alternative splice exon at nucleotide 1143 encoding a 7 amino acid insert (SEQ ID NO:3), Figure 3. In addition, a long 3' untranslated sequence (3' UTR), (SEQ ID NO:4), was isolated and is shown in Figures 4A and 4B.

- 5 **[0078]** Using the GCG pileup program (Feng and Doolittle, 1987, *J. Mol. Biol.*, 25:351-360), the alignment between the various human KCNQ potassium channel proteins and hKCNQ1-5 was generated and is shown in Figures 5A-5C. The blackened areas represent identical amino acids among all 5 family members and the gray highlighted amino acids represent similar amino acids. Overall, the homology among the KCNQ family members shows between about 40-50% identity, with higher homology (e.g., about 60%) in the membrane-spanning domains and the pore regions.

Expression vectors

- 15 **[0079]** The present invention further encompasses expression vectors which comprise all or a portion of a nucleotide sequence encoding the hKCNQ5 protein/polypeptide described herein, or peptides thereof. Preferably, the expression vectors comprise all or a portion of the nucleic acid sequence as shown in SEQ ID NO:1.

- 20 **[0080]** Expression vectors are usually plasmids, but the invention includes other vector forms, such as viral vectors, as well as vectors that serve equivalent functions and become known in the art subsequently hereto. A person skilled in the art may also stably integrate a sequence encoding the KCNQ5 protein into the chromosome of an appropriate host cell using methods practiced in the art.

- 25 **[0081]** Expression vectors typically contain regulatory elements capable of affecting expression of the KCNQ5 protein. These regulatory elements can be heterologous or native KCNQ5 elements. Typically, a vector contains an origin of replication, a promoter, and a transcription termination sequence. The vector may also include other regulatory sequences, including mRNA stability sequences, which provide for stability of the expression product; secretory leader sequences, which provide for secretion of the expression
- 30

product; environmental feedback sequences, which allow expression of the structural gene to be modulated (e.g., by the presence or absence of nutrients or other inducers in the growth medium); marking sequences, which are capable of providing phenotypic selection in transformed host cells; restriction sites, which provide sites for cleavage by restriction endonucleases; and sequences which allow expression in various types of host cells, including prokaryotic cells, yeast, fungi, plant cells, insect cells, mammalian cells, including human cells and non-human animal cells, and cells of higher eukaryotes.

5 [0082] As will be appreciated by the skilled practitioner, expression vectors comprise a nucleic acid sequence encoding at least one KCNQ5 polypeptide, as described herein, operably linked to at least one regulatory sequence or element. Operably linked is intended to mean that the nucleotide acid sequence is linked to a regulatory sequence in a manner
10 which allows expression of the nucleotide sequence. Regulatory sequences are known in the art and are selected to direct expression of the desired protein in an appropriate host cell. Accordingly, the term regulatory sequence includes promoters, enhancers and other expression control elements (see D.V. Goeddel, 1990, Methods Enzymol., 185:3-7). It should
15 be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transfected and/or the type of protein desired to be expressed.

[0083] Vectors can contain one or more replication and inheritance systems for cloning or expression, one or more markers for selection in the
20 host, e.g. antibiotic resistance, and one or more expression cassettes. The inserted coding sequences can be synthesized by standard methods, isolated from natural sources, or prepared as hybrids. Ligation of the coding sequences to transcriptional regulatory elements (e.g., promoters, enhancers, and/or insulators) and/or to other amino acid encoding
25 sequences can be carried out using established methods.

[0084] An expression vector according to this invention is at least capable of directing the replication, and preferably the expression, of the nucleic acids and protein of this invention. Suitable origins of replication include, for example, the Col E1, the SV40 viral and the M13 origins of replication.

5 Suitable promoters include, for example, the cytomegalovirus (CMV) promoter, the lacZ promoter, the gal10 promoter and the Autographa californica multiple nuclear polyhedrosis virus (AcMNPV) polyhedral promoter. Suitable termination sequences include, for example, the bovine growth hormone, SV40, lacZ and AcMNPV polyhedral polyadenylation
10 signals. Examples of selectable markers include neomycin, ampicillin, and hygromycin resistance and the like. Specifically-designed vectors allow the shuttling of DNA between different host cells, such as bacteria-yeast, or bacteria-animal cells, or bacteria-fungal cells, or bacteria invertebrate cells.

[0085] KCNQ5 protein-encoding DNA of the present invention may be
15 inserted into several commercially available vectors. Nonlimiting examples include plasmid vectors compatible with mammalian cells, such as pUC, pBluescript (Stratagene, La Jolla, CA), pET (Novagen, Inc., Madison, WI), pREP (Invitrogen Corp.), pcDNA3 (Invitrogen), pCEP4 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-
20 neo (ATTC No. 37593), pBPV-1(8-2) (ATCC No. 37110), pDBPV-MMTneo(342-12) (ATCC No. 37224), pRSVgpt (ATCC No. 37199), pRSVneo (ATCC No. 37198), pSV2-dhfr (ATCC No. 37146), pUCTag (ATCC No. 37460), IZD35 (ATCC No. 37565), pLXIN and pSIR (Clontech) and pIRES-EGFP (Clontech). Baculovirus vectors such as pBlueBac,
25 BacPac™ Baculovirus Expression System (CLONTECH), and MaxBac™ Baculovirus Expression System, insect cells and protocols (Invitrogen) are available commercially and may also be used to produce high yields of biologically active protein. (See also, Miller, L.A. et al., 1993, Curr. Op. Genet. Dev., 3:97 and O'Reilly, D.R. et al., Baculovirus Expression Vectors:
30 A Laboratory Manual, p.127. In addition, prokaryotic vectors such as pcDNA2; and yeast vectors such as pYes2 are nonlimiting examples of

other vectors suitable for use with the present invention. For vector modification techniques, see J. Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

5 Host cells

[0086] Host cells containing an expression vector that comprises a nucleic acid sequence encoding the KCNQ5 protein of the present invention can be cultured under conditions suitable for the expression and recovery of the expressed protein, e.g., from cell membranes or cell lysates, using methods known and practiced by those in the art. The host cells preferably contain an expression vector which comprises all or a portion of the DNA sequence having the nucleotide sequence substantially as shown in SEQ ID NO:1, particularly the KCNQ5 coding region thereof. Suitable host cells include both prokaryotic cells (e.g., *E. coli* strains HB101, DH5a, XL1 Blue, Y1090 and JM101), plant cells, fungal cells, and eukaryotic cells. Eukaryotic recombinant host cells are preferred. Examples of eukaryotic host cells include, but are not limited to, yeast, e.g., *S. cerevisiae* cells, cell lines of human, bovine, porcine, monkey, and rodent origin, as well as insect cells, including but not limited to, *Spodoptera frugiperda* insect cells and *Drosophila*-derived insect cells. Mammalian species-derived cell lines suitable for use and commercially available include, but are not limited to, L cells, CV-1 cells, CHO cells, (CHO-K1, ATCC CCL 61), COS-1 cells (ATCC CRL 1650), COS-7 cells (ATCC CRL 1651), HEK 293 cells, human skin fibroblasts, 3T3 cells (ATCC CCL 92), HeLa cells (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

[0087] The expression vectors may be introduced into host cells by various methods known in the art. Exemplary, yet nonlimiting, methods include transfection by calcium phosphate precipitation, electroporation, liposomal fusion/lipofection, transformation, transduction, protoplast fusion, direct DNA injection, nuclear injection, microparticle (e.g., colloidal gold) bombardment and viral or phage infection. The host cells are then cultured

under conditions permitting expression of large amounts of KCNQ5 protein. Preferably, the cells containing expression vectors and expressing the KCNQ5 protein are clonally propagated and individually analyzed to determine the level of novel KCNQ5 potassium channel production.

- 5 **[0088]** Recombinant host cells expressing the KCNQ5 protein can be identified by any of six general and nonlimiting approaches: (1) DNA-DNA hybridization with probes complementary to the sequence encoding KCNQ5 protein (Southern blotting); (2) detection of marker gene functions, such as thymidine kinase activity, resistance to antibiotics, and the like (A marker
- 10 gene can be placed in the same plasmid as the KCNQ5 sequence under the regulation of the same or a different promoter); (3) detection of mRNA transcripts by hybridization assays (e.g., Northern blotting or a nuclease protection assay using a probe complementary to the RNA sequence); (4) immunodetection of gene expression (e.g., by Western blotting with antibody
- 15 to KCNQ5 protein); (5) detection of potassium channel activity, such as by patch-clamp analysis, radioisotope (e.g., ⁸⁶Rb) efflux, two electrode voltage clamp, or membrane potential-sensitive reagents (e.g., Dibac from Molecular Probes International); and (6) PCR with primers homologous to expression vector sequences or sequences encoding KCNQ5 protein. The
- 20 PCR produces a DNA fragment of predicted length, indicating incorporation of the expression system in the host cell. DNA sequencing may be performed by various known methods. See, for example, the dideoxy chain termination method in Sanger et al., 1977, Proc. Natl. Acad. Sci. U.S.A., 74: 5463-7 and the Maxam-Gilbert method in Maxam-Gilbert, 1977, Proc. Natl.
- 25 Acad. Sci. U.S.A., 74: 560-4.

- [0089]** The KCNQ5 polypeptide of the present invention can be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate isolation and protein purification. Nonlimiting examples of protein purification facilitating domains include metal chelating peptides,
- 30 such as histidine-tryptophan modules that allow purification on immobilized beads (J. Porath, 1992, Protein Exp. Purif. 3:263); protein A domains that

allow purification on immobilized immunoglobulin; and the FLAGS domain extension/affinity purification system (Immunex Corp.). The inclusion of a cleavable linker sequence, such as Factor XA, or enterokinase (Invitrogen), between the purification domain and the potassium channel coding region is also useful to facilitate purification of the expressed KCNQ5 polypeptide.

[0090] Further, a host cell strain may be selected for its ability to modulate the expression of the inserted and expressed sequences, or to process the expressed protein in a desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a nascent form of the protein may also be important for correct folding, insertion and/or function. Different host cells, such as CHO, HeLa, MDCK, 293 (ATCC CRL 1573), WI38, NIH 3T3, HEK293, and the like, have specific cellular machinery and characteristic mechanisms for such post-translational activities, and may be employed to ensure the correct modification and processing of the introduced, heterologous protein.

[0091] Examples of protocols useful for detecting and measuring the expression of the novel KCNQ5 potassium channel polypeptide using either polyclonal or monoclonal antibodies include, but are not limited to, enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal antibody-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes may be utilized. Competitive binding techniques may also be employed (See, for example, R. Hampton, 1990, Serological Methods – A Laboratory Manual, APS Press, St. Paul, MN and D.E. Maddox et al., 1983, J. Exp. Med., 158:1211).

[0092] As will be appreciated by those having skill in the art, the host cells of this invention can be employed in a variety of ways that are now apparent. For example, the cells can be used to screen for compounds that bind to or otherwise modulate or regulate the function of the KCNQ5 protein,

or at least one subunit thereof, which would be useful for modulation, for example activation, of KCNQ5 protein activity; to study signal transduction mechanisms and protein-protein interactions; and to prepare KCNQ5 protein for the uses as further described below. Membrane preparations from cells
5 transfected with vectors harboring the KCNQ5 nucleic acid sequence, or a functional portion thereof, wherein the KCNQ5 potassium channel polypeptide, or a portion thereof, is expressed in the membranes, can be used for screening compounds, for example as described in the protocols of international application WO 99/31232.

10 **[0093]** Not all expression vectors and DNA regulatory sequences will function equally well to express the DNA sequences of this invention. Neither will all host cells function equally well with the same expression system. However, one of ordinary skill in the art may make a selection among expression vectors, DNA regulatory sequences, and host cells using
15 the guidance provided herein without undue experimentation and without departing from the scope of the invention, to achieve expression and purification of the KCNQ5 protein for the variety of uses described.

KCNQ5 Polypeptide

[0094] Another embodiment of the present invention encompasses
20 polypeptides comprising all or a portion of the amino acid sequence of the hKCNQ5 protein (SEQ ID NO:2). Where a portion of the hKCNQ5 protein is used, the portion preferably exhibits potassium (K^+) channel activity or can be modulated to exhibit K^+ channel activity. In addition, and within the scope of the invention, are polypeptides that comprise all or a portion of
25 KCNQ5 that may contain one or more mutations so that the protein(s) fails to exhibit K^+ channel activity, but can be used to screen for compounds that will activate the protein or portion thereof.

[0095] These KCNQ5 polypeptides may be prepared by methods known in the art. For example, chemical synthesis, such as the solid phase
30 procedure described by Houghton et al., 1985, Proc. Natl. Acad. Sci. U.S.A., 82: 5131-5, can be used. Another method is *in vitro* translation of mRNA. A

preferred method involves the recombinant production of protein in host cells as described above. For example, DNA comprising all or a portion of SEQ ID NO:1 can be synthesized by PCR as described above, inserted into an expression vector, and a host cell transformed with the expression
5 vector. Thereafter, the host cell is cultured to produce the desired polypeptides, which are isolated and purified. Protein isolation and purification can be achieved by any one of several known techniques; for example and without limitation, ion exchange chromatography, gel filtration chromatography and affinity chromatography, high pressure liquid
10 chromatography (HPLC), reversed phase HPLC, preparative disc gel electrophoresis.

[0096] In addition, cell-free translation systems (see J. Sambrook *et al.*, *supra*) can be used to produce recombinant KCNQ5 polypeptides or peptides. Suitable cell-free expression systems for use in accordance with
15 the present invention include rabbit reticulocyte lysate, wheat germ extract, canine pancreatic microsomal membranes, *E. coli* S30 extract, and coupled transcription/translation systems (Promega Corp., Madison, WI). These systems allow the expression of recombinant polypeptides or peptides upon the addition of cloning vectors, DNA fragments, or RNA sequences
20 containing coding regions and appropriate promoter elements.

[0097] As mentioned *supra*, protein isolation/purification techniques may require modification of the hKCNQ5 protein using conventional methods. For example, a histidine tag can be added to the protein to allow purification on a nickel column. Other modifications may cause higher or lower activity,
25 permit higher levels of protein production, or simplify purification of the protein. Such modifications may also help to identify specific KCNQ5 amino acids involved in binding, which, in turn, may assist in the rational drug design of KCNQ5 modulators. Amino acid substitutions can be made based on similarities in polarity, charge, solubility, hydrophobicity, hydrophilicity
30 and/or the amphipathic nature of the residues involved. Preferably, the biological activity or functional activity of the potassium channel is retained.

For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine. Amino acids with uncharged polar head groups or nonpolar head groups having similar hydrophilicity values include the following: leucine, isoleucine,
5 valine; glycine, alanine; asparagine, glutamine; serine, threonine; phenylalanine and tyrosine.

[0098] Conservative substitutions of amino acids in the KCNQ5 polypeptide of the present invention may include the use of a chemically derivatized residue to replace a non-derivatized residue, with the proviso
10 that the derivatized polypeptide displays the desired biological activity. D-isomers, as well as other known derivatives, may also be substituted for the naturally-occurring amino acids. (See, for example, U.S. Patent No. 5,652,369, issued July 29, 1997). Preferably, conservative substitutions are made without altering the biological activity of the resulting polypeptide. All
15 of the above-described modified polypeptides are included within the scope of the present invention.

[0099] Accordingly, the present invention embraces variants of the human KCNQ5 potassium channel molecule. Preferred are variants having at least about 80-85% total amino acid sequence similarity to SEQ ID NO:2; more
20 preferred are variants having at least 90% total amino acid sequence similarity to SEQ ID NO:2; and most preferred are variants having at least 95% total amino acid sequence similarity to SEQ ID NO:2, or a biologically active fragment thereof. A variant of the KCNQ5 polypeptide may have an amino acid sequence that is different by one or more amino acid
25 substitutions, or it may include conservative changes, wherein a substituted amino acid has similar structural or chemical properties, for example, replacement of leucine with isoleucine. It may happen, although more rarely, that a variant has nonconservative changes, for example, the replacement of a glycine with a tryptophan. Similar minor variations may
30 also include amino acid deletions or insertions, or both. Those having skill in the art will appreciate that guidance in determining which and how many

amino acid residues may be substituted, inserted, or deleted without abolishing or diminishing biological or immunological activity may be obtained using computer programs known and used in the art, such as DNASTar software.

5 **[0100]** Also contemplated according to the present invention are a number of other variations of the above-described polypeptides. Such variations include salts and esters of the polypeptides, as well as precursors of the aforementioned polypeptides (e.g., having N-terminal substituents such as methionine, N-formylmethionine and leader sequences). The present
10 invention is intended to embrace all such variations.

[0101] As will be described further *infra*, the hKCNQ5 polypeptide, and peptides thereof, of the present invention can be utilized in a wide variety of ways. For example, one may use them to generate polyclonal or
monoclonal antibodies by techniques known and practiced in the art. The
15 antibodies can be used for immunodetection (e.g., radioimmunoassay, enzyme immunoassay, or immunocytochemistry), immunopurification (e.g., affinity chromatography) of polypeptides from various sources, or immunotherapy (i.e., for potassium channel inhibition or activation). Further, the KCNQ5 polypeptide, or peptide fragments thereof, can be used in
20 screening or binding assays.

Functional Expression

[0102] Biologically active hKCNQ5 mRNA can be introduced into host cells, either heterologous or homologous to the vector or polynucleotide molecule, for functional expression and analyses by methods well-known in
25 the art. Synthetic mRNA from vector constructs as described herein, for example, may be injected into *Xenopus* oocytes for functional expression analysis (Goldin, A., 1992, Methods Enzymol., 207:266). Expressed hKCNQ5 potassium channels can be examined using standard two-electrode voltage clamp techniques (See, for example, Stuhmer W., 1992, Methods Enzymol., 207:319; and Kohler et al., 1996, Science, 273:1709).
30 Potassium concentrations inside the cell may be altered, for example, by

adding a potassium ionophore, or by co-expression with a receptor that causes a rise in intracellular potassium. Alternatively, potassium concentrations may be altered by pulling inside-out patches and changing potassium concentrations in the bath medium (See, for example, Grissmer, S. et al., 1993, J. Gen. Physiol., 102:601). Standard biophysical parameters, such as activators, potassium dependence, single-channel conductance, inactivation, tail currents, potassium selectivity, and pharmacology of various K⁺ channel blockers, including TEA (Example 4), Apamin, and the like, may be tested (See, Grissmer, S. et al., 1993, J. Gen. Physiol., 102:601).

[0103] Alternatively, cRNA (i.e., synthetic RNA from a cDNA construct) can be introduced into host cells, such as *Xenopus* oocytes, as described above (Example 4), or mammalian cells, for example, RBL cells (ATCC CRL 1378) or 293 cells (ATCC CRL 1573), can be transformed using routine methods in the art. As an example, direct nucleic acid injection can be employed, such as the Eppendorf microinjection system (Micromanipulator 5171 and Transjector 5242). The injected/transformed cells can be analyzed for K⁺ currents about 4 hours later using patch-clamp techniques, which are commonly practiced in the art (S.R. Ikeda et al., 1992, Pflugers Arch., 422(2):201-203; and S. Grissmer et al., 1993, J. Gen. Physiol., 102:601).

Over-Expression of the Novel KCNQ5 Channel in Cell Lines

[0104] Transient and/or stably transfected cells, preferably eukaryotic cells, such as HEK cells, containing the KCNQ5 nucleic acid comprising the coding region, are envisioned for high level expression of the KCNQ5 potassium channel. Such eukaryotic transfectants are suitable for using in performing pharmacological target binding studies for the identification of molecules that open or block the novel KCNQ5 channel.

[0105] Transient expression of the KCNQ5 coding region can be achieved by direct transfection into mammalian cells using standard techniques (Omari, K. et al., 1997, J. Physiol., 499:369; Panyi, G. et al., 1996, J. Gen.

Physiol., 107(3):409). High level transient expression can be attained using viral systems commonly used in the art, e.g., vaccinia virus, baculovirus, or adenovirus. Channel numbers resulting from these systems are typically from 5 to 500K per cell (Kamb, A. et al., 1992, Methods Enzymol., 207:423; 5 Sun, T. et al., 1994, Biochemistry, 33(33):9992; and Spencer, R.H. et al., 1997, J. Biol. Chem., 272:2389).

[0106] Stable transfection of host cells using nucleic acid sequences, e.g., (SEQ ID NO:1), which encode the novel hKCNQ5 potassium channel described herein (SEQ ID NO:2), or biologically active variants or fragments 10 thereof, can be prepared using, for example, 3T3, L929, COS, HEK or CHO cells. A vector suitable for use in preparing stable transfectants is pcDNA/neo (Invitrogen).

[0107] An exemplary procedure for carrying out the transfection assays is as follows. Cells are grown to about 50% confluency in 60 mm tissue 15 culture plates in media and under conditions that are according to the requirements of the particular cell line. Cells are transfected or transformed with 5 µg of pure DNA comprising a coding region for the KCNQ5 potassium channel, e.g., SEQ ID NO:1, in a pcDNA/neo plasmid vector using the Lipofection reagent as described by the supplier (Life Technologies Gibco 20 BRL, Bethesda, MD). After transfection, the cells are incubated at 37°C for three days in medium containing 10% FCS. Cells are trypsinized, seeded onto 100 mm plates, and then selected with 300 µg/ml of G418 (neomycin). Only those cells which have stably integrated the heterologous coding region will grow in the presence of G418, since resistance is conferred by 25 the neomycin-resistance gene in the vector. Isolated clones are processed for about 2-3 rounds of selection/purification and are subjected to patch-clamp analysis for K⁺ currents.

[0108] The various cell lines that express or over-express the novel KCNQ5 encoded by the polynucleotide coding region, i.e., SEQ ID NO:1, or 30 functional fragment thereof, can be used in ligand binding assays to screen for pharmacologically active molecules that bind the KCNQ5 channel, or one

or more subunits thereof. For example, a radio-labeled ligand is used as a measurable displacement entity in a noncompetitive or competitive binding assay. Nonlimiting examples of ligands for use in such assays include TEA; 4-aminopyridine (4-AP), as well as 2-AP and 3-AP; 3,4- and 2,3-
5 diaminopyridine; BaCl₂; CsCl; strychnine; phencyclidine; pyridostigmine; 9-aminoacridine; DuP-996 (3,3-bis (4-pyridinylmethyl)-1-phenylindolin-2-one; linopiridine); clofilium; quinidine; aminoquinolines; and quinine. Examples of peptide toxins for use as ligands in such binding assays include, but are not limited to, stichodactylotoxin, apamin, charybdotoxin, kaliotoxin and
10 margotoxin.

Method for Detecting KCNQ5-Encoding Nucleic Acids

[0109] Another embodiment of the present invention provides a method for detecting nucleic acids encoding the KCNQ5 protein. In this method, nucleic acids of unknown sequence are contacted with a nucleic acid having
15 a sequence complementary to a known coding sequence (e.g., a sequence of at least about 10 nucleotides from, e.g., SEQ ID NO:1, particularly the coding region thereof), wherein the latter nucleic acid has a detectable marker; and (b) the presence of marker bound to any of the nucleic acids of unknown sequence is determined. The presence of bound marker indicates
20 the presence of the desired nucleic acids. This method can be applied to detect KCNQ5 nucleic acid in other tissues (which may have different regulatory elements) and nucleic acids from other species (e.g., monkey).

[0110] Nucleic acids for analysis by this method can be obtained using commonly practiced and routine methods in the art. For genomic DNA, a
25 tissue sample can be rapidly frozen and then crushed into readily digestible pieces, which are incubated in an enzyme, such as proteinase K, and SDS to degrade most of the cellular proteins. The genomic DNA is then deproteinized by successive phenol/chloroform/isoamyl alcohol extractions, recovered by ethanol precipitation, dried and resuspended in buffer. For
30 RNA, cultured cells are lysed in 4M guanidinium solution and the lysate is drawn through a 20-gauge needle. The RNA is applied to a cesium chloride

step gradient and pelleted by centrifugation and the supernatant is removed. The pellet should contain purified RNA.

[0111] The detectable marker may be a radioactive ion linked to one of the nucleotides of the complementary nucleic acid. Common radioactive labels are ^{32}P and ^{35}S , although other labels, such as biotin-avidin, may be used. Those having skill in the art are aware of various methods to attach the labels to the complementary nucleic acid (e.g., the random primer method for attachment of ^{32}P or ^{35}S).

[0112] Methods of detecting nucleic acids are generally known by those having ordinary skill in the art. For example, one may perform a Southern or Northern blotting procedure using a radiolabeled KCNQ5 complementary oligonucleotide probe. Hybridization is then detected by autoradiography. Depending on the marker, other detection methods (e.g., spectrophotometry and non-radioactive labels, such as chemiluminescence or fluorescence or enzymes).

Methods for Detecting KCNQ5 Protein Modulators/Screening Assays

[0113] The present invention is further directed to methods for detecting modulators of the KCNQ5 protein described herein. A screen for KCNQ5 protein modulators entails detecting binding of molecules (e.g., polypeptides, natural products, synthetic compounds) in cells expressing KCNQ5 protein.

[0114] The cloning and sequencing of the hKCNQ5 polynucleotide provides the ability to generate recombinant host cells useful in expressing all or a portion of the KCNQ5 protein allowing for screening of natural products and synthetic compounds that bind to and/or modulate KCNQ5 protein activity. A process for detecting KCNQ5 protein modulators requires transforming a suitable vector into compatible host cells as described *supra*.

Transformed cells are then treated with test substances (e.g., synthetic compounds or natural products), and channel activity is measured and/or assessed in the presence and absence of the test substance.

[0115] More specifically, compounds that modulate KCNQ5 activity may be DNA, RNA, peptides, proteins, or non-protein organic molecules. Such

- compounds may modulate channel activity by increasing or attenuating the expression of DNA or RNA which encodes the potassium channel, or may antagonize or agonize the biological activity of the novel KCNQ5 channel itself. The assays to detect compounds that modulate the expression of
- 5 DNA or RNA encoding hKCNQ5 may be a simple "yes/no" assay to qualitatively determine if there is a change in expression or function. Alternatively, the assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample.
- 10 **[0116]** The human KCNQ5 protein described herein, or immunogenic fragments or oligopeptides thereof, can be used for screening therapeutic drugs or compounds in a variety of drug screening techniques. The fragment employed in such a screening assay may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly.
- 15 The reduction or abolition of activity of the formation of binding complexes between the potassium channel protein and the agent being tested can be measured. Thus, the present invention provides a method for screening or assessing a plurality of compounds for their specific binding affinity with the KCNQ5 potassium channel polypeptide, or a bindable portion thereof, e.g. a
- 20 subunit, comprising providing a plurality of compounds, combining the KCNQ5 polypeptide, or a bindable fragment thereof, with each of a plurality of compounds for a time sufficient to allow binding under suitable conditions and detecting binding of the KCNQ5 polypeptide to each of the plurality of test compounds, thereby identifying the compounds that specifically bind to
- 25 the KCNQ5 polypeptide.
- [0117]** Methods of identifying compounds that modulate the activity of the hKCNQ5 potassium channel polypeptide are provided by the present invention and comprise combining a potential or candidate compound or drug modulator of a potassium channel biological activity with a KCNQ5
- 30 polypeptide, preferably having the amino acid sequence set forth in SEQ ID NO:2, and measuring an effect of the candidate compound or drug

modulator on the biological activity of the KCNQ5 polypeptide. Such measurable effects include, for example, physical binding interaction; the ability to pass K⁺ ions, or other ions used for screening; effects on native and cloned KCNQ5 cell line membrane potential, effects on neurotransmitter
5 release, and effects of modulators or other KCNQ5-mediated neurophysiological measures.

[0118] Another method of identifying compounds that modulate the biological activity of the hKCNQ5 potassium channel polypeptide comprises combining a potential or candidate compound or drug modulator of a
10 potassium channel biological activity with a host cell that expresses a KCNQ5 polypeptide, preferably having the amino acid sequence set forth in SEQ ID NO:2, and measuring an effect of the candidate compound or drug modulator on the biological activity of the KCNQ5 polypeptide. The host cell can also be capable of being induced to express the KCNQ5 polypeptide,
15 e.g., via inducible expression. Physiological effects of a given modulator candidate on the KCNQ5 potassium channel polypeptide can also be measured. Thus, cellular assays for KCNQ5 modulators may be either direct measurement or quantification of the physical biological activity of the potassium channel, or may be measurement or quantification of a
20 physiological or a neurological effect. Such methods preferably employ the hKCNQ5 potassium channel polypeptide described herein, or overexpressed recombinant KCNQ5 potassium channel polypeptide in suitable host cells containing an expression vector as described herein, wherein the KCNQ5 potassium channel polypeptide is expressed,
25 overexpressed, or undergoes upregulated expression.

[0119] Another aspect of the present invention embraces a method of screening for a compound that is capable of modulating the biological activity of the KCNQ5 polypeptide, comprising providing a host cell containing an expression vector harboring a nucleic acid sequence encoding
30 the KCNQ5 polypeptide (SEQ ID NO:2), or a functional portion thereof; determining the biological activity of the expressed KCNQ5 polypeptide in

the absence of a modulator compound; contacting the cell with the modulator compound and determining the biological activity of the expressed KCNQ5 polypeptide in the presence of the modulator compound.

In such a method, a difference between the activity of the KCNQ5 polypeptide in the presence of the modulator compound and in the absence of the modulator compound indicates a modulating effect of the compound.

[0120] In another of its aspects, the present invention encompasses screening and small molecule (e.g., drug) detection assays which involve the detection or identification of small molecules that can bind to a given protein, i.e., the KCNQ5 protein. Particularly preferred are assays suitable for high throughput screening methodologies. In such binding-based screening or detection assays, a functional assay is not typically required. All that is needed is a target protein, preferably substantially purified, and a library or panel of compounds (e.g., ligands, drugs, small molecules) to be screened or assayed for binding to the protein target. Preferably, most small molecules that bind to the target protein will modulate activity in some manner, due to preferential, higher affinity binding to functional areas or sites on the protein.

[0121] An example of such an assay is the fluorescence based thermal shift assay (3-Dimensional Pharmaceuticals, Inc., 3DP, Exton, PA) as described in U.S. Patent Nos. 6,020,141 and 6,036,920 to Pantoliano et al.; see also, J. Zimmerman, 2000, *Gen. Eng. News*, 20(8)). The assay allows the detection of small molecules (e.g., drugs, ligands) that bind to expressed, and preferably purified, KCNQ5 protein based on affinity of binding determinations by analyzing thermal unfolding curves of protein-drug or ligand complexes. The drugs or binding molecules determined by this technique can be further assayed, if desired, by methods, such as those described herein, to determine if the molecules affect or modulate function or activity of the target protein.

[0122] To purify a KCNQ5 potassium channel polypeptide to measure a biological binding or ligand binding activity, the source may be a whole cell

lysate that can be prepared by successive freeze-thaw cycles (e.g., one to three) in the presence of standard protease inhibitors. The hKCNQ5 potassium channel may be partially or completely purified by standard protein purification methods, e.g., affinity chromatography using specific
5 antibody described *infra*, or by ligands specific for an epitope tag engineered into the recombinant KCNQ5 polypeptide molecule, also as described herein. Binding activity can then be measured as described.

[0123] Compounds which are identified according to the methods provided herein, and which modulate or regulate the biological activity or physiology
10 of the KCNQ5 potassium channel according to the present invention are a preferred embodiment of this invention. It is contemplated that such modulatory compounds may be employed in treatment methods for treating a condition that is mediated by the novel hKCNQ5 potassium channel by administering to an individual in need of such treatment a therapeutically
15 effective amount of the compound identified by the methods described herein.

[0124] In addition, the present invention provides methods for treating an individual in need of such treatment for a condition that is mediated by the hKCNQ5 potassium channel of the invention, or by a neuroaffective,
20 neurophysiological, or neuropsychological disorder or condition, such as described herein, comprising administering to the individual a therapeutically effective amount of the hKCNQ5 channel-modulating compound identified by a method provided herein.

Chimeric or Fusion Proteins involving hKCNQ5

25 [0125] In another embodiment of the present invention, a nucleic acid sequence which encodes a hKCNQ5 potassium channel molecule substantially as depicted in SEQ ID NO:2, or a biologically active fragment thereof, can be ligated to a heterologous sequence to encode a fusion protein (also called a chimeric protein). For example, for screening
30 compounds for modulating biological activity of hKCNQ5, it may be useful to encode a chimeric potassium channel molecule as described herein for

expression in host cells.

- [0126]** Chimeric constructs may also be used to express a "bait", according to methods well known using a yeast two-hybrid system, to identify accessory native peptides that may be associated with the novel KCNQ5 potassium channel molecule described herein. (Fields, S. et al., 1995, Trends Genet., 10:286; Allen, J.B. et al., 1995, TIBS, 20:511). A yeast two-hybrid system has been described wherein protein:protein interactions can be detected using a yeast-based genetic assay via reconstitution of transcriptional activators. (Fields, S. and Song, O., 1989, Nature, 340:245). The two-hybrid system involves the ability of a pair of interacting proteins to bring a transcription activation domain into close proximity with a DNA-binding site that regulates the expression of an adjacent reporter gene. Commercially available systems such as the Clontech Matchmaker™ systems and protocols (Clontech, Palo Alto, CA) may be used with conjunction with the present invention. (See also, Mendelsohn, A.R. and Brent, R., 1994, Curr. Op. Biotech., 5:482; Phizicky, E.M. and Fields, S., 1995, Microbiological Rev., 59(1):94; Yang, M. et al., 1995, Nucleic Acids Res., 23(7):1152; Fields, S. and Sternglanz, R., 1994, TIG, 10(8):286; and U.S. Patent No. 6,283,173 and 5,468,614).
- [0127]** Modified screening systems, for instance, can be practiced either with a positive readout or with a negative readout such as that in the recently developed versions of "Reverse Y2H" approach. (See, for example, Vidal M. et al., 1996, Proc. Natl. Acad. Sci. U.S.A., 17;93(19):10321-10326; Vidal M., et al., 1996, Proc. Natl. Acad. Sci. U.S.A., 17;93(19):10315-10320; White M.A., 1996, Proc. Natl. Acad. Sci. U.S.A., 17;93(19):10001-10003; and Leanna C.A. and Hannink M., 1996, Nucleic Acids Res., 1;24(17):3341-3347).

Antibodies

- [0128]** Polyclonal antibodies that are immunoreactive with (and monospecific for) the KCNQ5 polypeptide, or an immunoreactive fragment thereof, can be purified from antisera of an animal previously immunized

with the KCNQ5 polypeptide, or immunoreactive fragment thereof, as immunogen. In addition, monoclonal antibodies can be prepared using protocols and techniques routinely practiced in the art (e.g., Kohler and Milstein, 1975, Nature, 256:495).

- 5 **[0129]** Nonlimiting examples of peptide fragments or portions of the KCNQ5 polypeptide for use as immunogens, or in methods as described herein, include the following: peptide fragment 1 ends at amino acid 51 of the KCNQ5 polypeptide sequence and comprises the sequence GGGGGGLRESRRGKQGAR (SEQ ID NO:5); peptide fragment 2 ends at
- 10 amino acid 80 of the KCNQ5 polypeptide sequence and comprises the sequence PLSYTSSQSCRRNVKYRRVQNYL (SEQ ID NO:6); peptide fragment 3 ends at amino acid 166 and comprises the sequence CRYRGWQGRLRFARKP (SEQ ID NO:7); peptide fragment 4 ends at amino acid 262 of the KCNQ5 polypeptide sequence and comprises the sequence
- 15 VEKDANKEFST (SEQ ID NO:8); peptide fragment 5 ends at amino acid 335 of the KCNQ5 polypeptide sequence and comprises VQEQHRQKHFEKRRNPA (SEQ ID NO:9); polypeptide fragment 6 ends at amino acid 453 of the KCNQ5 polypeptide sequence and comprises the sequence
- 20 HTCSPTKKEQGEASSSQKLSFKERVMA SPRGQSIKSRQASVGDRRSPS TDITAEGSPTKVQKSWSFNDRTFRPSLRLKSSQPKPVI (SEQ ID NO:10); peptide fragment 7 ends at amino acid 471 of the KCNQ5 polypeptide sequence and comprises the sequence LGTDDVYDEKGCQ (SEQ ID NO:11); peptide fragment 8 ends at amino acid 511 of the KCNQ5
- 25 polypeptide sequence and comprises the sequence HVAKRKFKETLRPYDV (SEQ ID NO:12); peptide fragment 9 ends at amino acid 564 of the KCNQ5 polypeptide sequence and comprises the sequence LGKGQITSDKKSREKITA EHETTDD (SEQ ID NO:13); peptide fragment 10 ends at amino acid 585 of the KCNQ5 polypeptide sequence and comprises
- 30 the sequence KVEKQVQSIESKL (SEQ ID NO:14); peptide fragment 11 ends at amino acid 652 of the KCNQ5 polypeptide sequence and comprises

the sequence

PPFECEQTSDYQSPVDSKDLGSAQNSGCLSRSTSANISRG (SEQ ID

NO:15); peptide fragment 12 ends at amino acid 709 of the KCNQ5

polypeptide sequence and comprises the sequence TIANQINTAPKPAA

5 (SEQ ID NO:16); peptide fragment 13 ends at amino acid 738 of the KCNQ5

polypeptide sequence and comprises the sequence KHLPRPETLHPNPAGL

(SEQ ID NO:17); peptide fragment 14 ends at amino acid 777 of the KCNQ5

polypeptide sequence and comprises the sequence

SKENVQVAQSNLTKDRSMRKSFDMMGG (SEQ ID NO:18); peptide fragment

10 15 ends at amino acid 833 of the KCNQ5 polypeptide sequence and

comprises the sequence LSGSESSGSRGSQDFYPKWRESK (SEQ ID

NO:19); peptide fragment 16 ends at amino acid 860 of the KCNQ5

polypeptide sequence and comprises the sequence

EEVGPEETETDTFDAAPQPARE (SEQ ID NO:20); and peptide fragment 17

15 ends at amino acid 880 of the KCNQ5 polypeptide sequence and comprises

the sequence DSLRTGRSRSSQSIC (SEQ ID NO:21).

[0130] As used herein, "antibody" or "antibodies" refers to intact molecules as well as fragments thereof, such as Fab, F(ab)₂, and Fv, which are

capable of binding an epitopic determinant of the KCNQ5 immunogen. As

20 will be appreciated by those having skill in the art, the immunogen can be

conjugated to a carrier protein, if desired, to increase immunogenicity,

particularly, if a small peptide or fragment of the KCNQ5 polypeptide is

used. Commonly used carriers that are routinely chemically coupled to

peptides include serum albumins, i.e., bovine, sheep, goat, or fish serum

25 albumin; thyroglobulin; and keyhole limpet hemocyanin. The coupled

immunogen-carrier is then used to immunize a recipient animal (e.g.,

mouse, rat, guinea pig, sheep, goat, or rabbit).

[0131] The term "antigenic determinant" refers to that fragment of a molecule (i.e., an epitope) that makes contact with a particular antibody.

30 When an isolated and/or purified KCNQ5 polypeptide is used to immunize a host animal, numerous regions of the polypeptide may induce the production

of antibodies which bind specifically to a given region or three-dimensional structure on the polypeptide; these regions or structures are referred to as antigenic determinants or epitopes. An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

[0132] The antibodies can be elicited in an animal host by immunization with KCNQ5-derived immunogenic components, or can be formed by *in vitro* immunization (sensitization) of immune cells. The antibodies can also be produced in recombinant systems transformed, transfected, infected or transduced with appropriate antibody-encoding DNA. Alternatively, the antibodies can be constructed by biochemical reconstitution of purified heavy and light chains. Antibodies embraced by the present invention include hybrid antibodies, chimeric antibodies, humanized antibodies (see, for example, U.S. Patent No. 5,585,089 to C.J. Queen et al.) and univalent antibodies. Using such antibodies, for example, KCNQ5 polypeptide, or an immunogenic fragment or portion thereof, can be detected in a test sample by chromatography on antibody-conjugated solid-phase matrices or supports (see E. Harlow and D. Lane, 1999, *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), or by immunoassay. Preferred are antibodies that specifically recognize and bind to KCNQ5.

[0133] Thus, according to the present invention, antibodies can be generated that are specific for the full-length KCNQ5 molecule, or immunoreactive portions or fragments thereof. The antibodies can be employed to prepare KCNQ5 potassium channel antibody affinity columns. For example, gel supports or beads can be activated with various chemical compounds, e.g., cyanogen bromide, N-hydroxysuccinimide esters, and antibodies can be bound thereto. More particularly and by way of example, anti-KCNQ5 potassium channel antibodies can be added to Affigel-10 (Biorad), a gel support which is activated with N-hydroxysuccinimide esters, such that the antibodies form covalent linkages with the agarose gel bead

support. The antibodies are then coupled to the gel via amide bonds with a spacer arm. The remaining activated esters are then quenched with ethanolamine HCl, 1M, pH 8. The column is washed with water, followed by 0.23M glycine HCl, pH 2.6, to remove any non-conjugated antibody or
5 extraneous protein. The column is then equilibrated in phosphate buffered saline (PBS), (pH 7.3) with appropriate detergent, and the sample materials, i.e., cell culture supernatants or cell extracts, for example, containing hKCNQ5 polypeptide (prepared using appropriate membrane solubilizing surfactants) are slowly passed over the column. The column is washed with
10 PBS/surfactant until the optical density falls to background. The protein is then eluted from the column with 0.23M glycine-HCl, pH 2.6/surfactant. The purified hKCNQ5 polypeptide is then dialyzed against PBS/surfactant.

[0134] Recombinant hKCNQ5 potassium channel molecules can be separated from cellular proteins by use of an immunoaffinity column made
15 with monoclonal or polyclonal antibodies that are specific for either full-length hKCNQ5 potassium channel polypeptide, or peptide fragments of hKCNQ5.

[0135] In addition, the KCNQ5 potassium channel polypeptide of the present invention can be used to affinity purify biological effectors from
20 biological materials, e.g., disease tissue or cells, using affinity chromatography techniques that are known and practiced by those having skill in the art. For example, a KCNQ5 potassium channel polypeptide, or a peptide or fragment thereof, is affixed to a solid matrix, e.g., CNBr-activated Sepharose, according to the protocol of the supplier (Pharmacia,
25 Piscataway, NJ). An homogenized and buffered cellular or tissue solution containing a potential biomolecule of interest is passed through the column. After washing, the column retains only the biological effector molecule, which is subsequently eluted from the column, e.g., using 0.5M acetic acid or a NaCl gradient.

30 Diagnostic Assays

[0136] Antibody titers of ascites or hybridoma culture fluids are determined

by various serological or immunological assays which include, but are not limited to, precipitation, passive agglutination, enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) techniques.

Similar diagnostic assays are used to detect the presence of the novel
5 KCNQ5 potassium channel biomolecule in body fluids, tissue and cell extracts, or membrane preparations.

[0137] Diagnostic assays using antibodies immunoreactive with the human KCNQ5 potassium channel polypeptide are useful for the diagnosis of conditions, disorders or diseases characterized by the abnormal
10 expression of the hKCNQ5 protein, or by the expression of genes associated with abnormal cell growth or abnormal neurophysiology. Diagnostic assays for the hKCNQ5 biomolecule of this invention include methods utilizing an antibody and a label to detect the human KCNQ5 potassium channel polypeptide in human body fluids, cells, tissues or
15 sections or extracts or membrane preparations of such tissues. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, the polypeptides and antibodies are labeled by conjugating them, either covalently or noncovalently, with a reporter molecule, a vast number and type of which are well-known to those
20 skilled in the art.

[0138] A variety of protocols for measuring the KCNQ5 potassium channel polypeptide, using either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples include ELISA, RIA and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based
25 immunoassay utilizing monoclonal antibodies reactive with two non-interfering epitopes on the hKCNQ5 potassium channel polypeptide is preferred, but a competitive binding assay can also be employed. These assays are described in a number of publications available to the skilled practitioner, among them: Maddox, D.E. et al., 1983, J. Exp. Med.,
30 158:1211; Sites, D.P. et al., 1, *Basic and Clinical Immunology*, Ch. 22, 4th Ed., Lange Medical Publications, Los Altos, CA (1982); U.S. Patent Nos.

3,654,090; 3,850,752; and 4,016,043.

[0139] In order to provide a basis for the diagnosis of disease, normal or standard values for the human KCNQ5 potassium channel polypeptide expression are preferably established. This is accomplished by combining
5 body fluids, cell extracts, or cell membrane preparations from normal subjects, either animal or human, with antibody to the hKCNQ5 polypeptide under conditions suitable for complex formation; such conditions are well known in the art. The amount of standard complex formation may be quantified by comparing it with a dilution series of positive controls, where a
10 known amount of antibody is combined with known concentrations of purified potassium channel polypeptide. Then, standard values obtained from the normal samples may be compared with values obtained from samples from subjects potentially affected by a disorder or disease related to/associated with hKCNQ5 channel biomolecule expression. Deviation
15 between standard and subject values establishes the presence of the disease state.

[0140] Moreover, kits containing KCNQ5 potassium channel nucleic acid, antibodies to KCNQ5 channel polypeptide or KCNQ5 protein may be prepared. Such kits can be used to detect heterologous nucleic acid which
20 hybridizes to the KCNQ5 potassium channel nucleic acid, or to detect the presence of KCNQ5 protein or peptide fragments in a sample. Such characterization is useful for a variety of purposes including, but not limited to, forensic analyses and epidemiological studies.

[0141] The DNA and RNA polynucleotide molecules, recombinant KCNQ5
25 protein and antibodies thereto, according to the present invention may be used to screen and measure levels of the novel potassium channel DNA, RNA or protein. The recombinant proteins, DNA and RNA polynucleotide molecules, and antibodies, allow the formulation of kits suitable for the detection and typing of the novel human KCNQ5 potassium channel
30 molecule. Such a kit would comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would

further comprise reagents such as recombinant KCNQ5 potassium channel or anti-KCNQ5 potassium channel antibodies suitable for detecting the novel KCNQ5 potassium channel biomolecule. The carrier may also contain a means for detection such as labeled antigen or enzyme substrates or the like. Suitable instructions for performing the assays as intended by the kits are also included therein.

[0142] Polynucleotide sequences which encode the novel hKCNQ5 potassium channel of the present invention may be used for the diagnosis of neuroaffective, neurophysiological, or neuropsychological conditions or diseases with which the expression and/or function of the novel KCNQ5 biomolecule is associated or linked. For example, polynucleotide sequences encoding the KCNQ5 potassium channel may be used in hybridization or PCR assays of fluids or tissues or membrane preparations from biopsies to detect the expression of the biomolecule. The form of such qualitative or quantitative methods may include Southern or Northern analysis, dot blot or a variety of other membrane-based technologies; PCR technologies; dip stick, pin, chip and ELISA technologies. All of these techniques are well known in the art and are the basis of many commercially available diagnostic kits. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or in monitoring the treatment of an individual patient. Once disease is established, a therapeutic agent is administered and a treatment profile is generated. Such assays may be repeated on a regular basis to evaluate whether the values in the profile progress toward or return to the normal or standard pattern. Successive treatment profiles may be used to show or monitor the efficacy of treatment over a period of several days or several months.

[0143] Polynucleotide sequences which encode the novel hKCNQ5 potassium channel may also be employed in analyses to map chromosomal locations, for example, to screen for functional association with disease markers. Moreover the sequences described herein are contemplated for

use to identify human sequence polymorphisms and possible association with disease, as well as for analyses to select optimal sequences from among possible polymorphic sequences for the design of compounds to modulate the biological activity, and therefore regulate physiological disorders, most preferably neuroaffective or neurophysiological disorders *in vivo*. In addition, the sequences are contemplated to be used as screening tools for use in the identification of appropriate human subjects and patients for therapeutic clinical trials.

Therapeutic Agents/Uses

- 10 **[0144]** The sense and antisense KCNQ5 nucleic acid molecules can also be used as therapeutic agents for KCNQ-related indications. Vectors can be designed and constructed to direct the synthesis of the desired DNA or RNA or to formulate the nucleic acid as described in the art.

Antisense Nucleic Acid Molecules

- 15 **[0145]** Several references describe the usefulness of antisense molecules. See, for example, Toulme and Helene, 1988, Gene 72:51-8; Inouye, 1988, Gene, 72:25-34; Uhlmann and Peyman, 1990, Chemical Reviews, 90: 543-584; Biotechnology Newswatch (January 15, 1996), p. 4; Robertson, 1997, Nature Biotechnology, 15:209; Gibbons and Dza, 1996, 20 Science, 272: 689-93. Antisense sequences can be designed based on genomic DNA and/or cDNA, 5' and 3' flanking control regions, other flanking sequences, intron sequences, and nonclassical Watson and Crick base pairing sequences used in the formation of triplex DNA. Such antisense molecules include antisense oligodeoxyribonucleotides, 25 oligoribonucleotides, oligonucleotide analogues, and the like, and may comprise at least about 15 to 25 bases.

- [0146]** Antisense molecules may bind noncovalently or covalently to the KCNQ5 DNA or RNA. Such binding could, for example, cleave or facilitate cleavage of KCNQ5 DNA or RNA, increase degradation of nuclear or 30 cytoplasmic mRNA, or inhibit transcription, translation, binding of transactivating factors, or pre-mRNA splicing or processing. Antisense

molecules may also contain additional functionalities that increase stability, transport into and out of cells, binding affinity, cleavage of the target molecule, and the like. All of these effects would decrease expression of KCNQ5 protein and thus make the antisense molecules useful as KCNQ5 protein modulators.

[0147] In addition, the KCNQ5 nucleic acid sequence depicted in SEQ ID NO:1 may be used to design antisense molecules/constructs to investigate the physiological relevance of this novel potassium channel in cells by knocking out or knocking down the expression of the endogenous gene. For down-regulating the expression of the novel KCNQ5 potassium channel of the present invention in mammalian cells, an antisense expression construct containing the complement DNA sequence to the sequence, or a portion thereof, essentially as depicted in SEQ ID NO:1 can be prepared, for example, using the pREP10 vector (Invitrogen Corp.). Transcripts are expected to inhibit translation of the wild-type KCNQ5 mRNA in cells transfected with the construct. Antisense transcripts are effective for inhibiting translation of the native gene transcript and are capable of inducing physiological effects, e.g., regulation of neurophysiological disorders described herein. Translation is most effectively inhibited by blocking the mRNA at a site at or near the initiation codon. Accordingly, oligonucleotides that are complementary to the 5'-terminal region of the KCNQ5 potassium channel mRNA transcript are preferred. Secondary or tertiary structure which might interfere with hybridization is potentially minimal in this region. Moreover, sequences that are too distant in the 3' direction from the initiation site can be less effective in hybridizing with the mRNA transcripts because of a "read-through" phenomenon in which the ribosome appears to unravel the antisense/sense duplex to permit translation of the message.

[0148] Oligonucleotides that are complementary to and hybridizable with any portion of the novel KCNQ5 potassium channel mRNA are contemplated for use according to the present invention. (See, for example,

U.S. Patent No. 5,639,595, issued June 17, 1997, wherein methods of identifying oligonucleotide sequences that display *in vivo* activity are described). As described therein, expression vectors containing random oligonucleotide sequences derived from previously known polynucleotides are transformed into cells. The cells are then assayed for a phenotype resulting from the desired activity of the oligonucleotide. Once cells with the desired phenotype have been identified, the sequence of the oligonucleotide having the desired activity can be identified. Identification may be accomplished by recovering the vector or by polymerase chain reaction (PCR) amplification and sequencing the region containing the inserted nucleic acid material, using established methods and protocols in the art.

[0149] Nucleotide sequences that are complementary to the novel hKCNQ5 potassium channel polypeptide encoding polynucleotide sequence can be synthesized for antisense therapy. These antisense molecules may be DNA, stable derivatives of DNA, such as phosphorothioates or methylphosphonates, RNA, stable derivatives of RNA such as 2'-O-alkylRNA, or other oligonucleotide mimetics. (See, for example, U.S. Patent No. 5,652,355, *Hybrid Oligonucleotide Phosphorothioates*, issued July 29, 1997, and U.S. Patent No. 5,652,356, *Inverted Chimeric and Hybrid Oligonucleotides*, issued July 29, 1997, which describe the synthesis and effect of physiologically-stable antisense molecules).

[0150] Peptide nucleic acid (PNA) refers to an antisense molecule or antigenic agent which comprises an oligonucleotide linked to a peptide backbone of amino acid residues, terminating in lysine. PNA typically comprise oligonucleotides of at least 5 nucleotides linked to amino acid residues. These small molecules stop transcript elongation by binding to their complementary strand of nucleic acid (P.E. Nielsen et al., 1993, *Anticancer Drug Des.*, 8:53-63). PNA may be pegylated to extend their lifespan in the cell where they preferentially bind to complementary single stranded DNA or RNA.

[0151] Potassium channel antisense molecules may be introduced into

cells by microinjection, liposome encapsulation or by expression from vectors harboring the antisense sequence, and the like. Antisense therapy may be particularly useful for the treatment of diseases where it is beneficial to modulate the biological activity of the hKCNQ5 potassium channel

5 described herein.

Delivery of hKCNQ5 Nucleic Acid to Cells

[0152] The human KCNQ5 potassium channel polypeptide-encoding nucleic acid described herein may be delivered to cells, either as naked DNA or in an expression vector, wherein the cells express the polypeptide. In this way, the KCNQ5 potassium channel polypeptide of the present invention can be delivered to the cells of target organs. Conversely, KCNQ5 potassium channel polypeptide antisense gene therapy may be used to modulate the expression of the polypeptide in the cells of target organs and thus regulate biological activity.

15 [0153] The potassium channel polypeptide coding region, or other suitable regions, e.g., the S4 or pore region coding regions, can be ligated into expression vectors, preferably, viral expression vectors, which mediate transfer of the transactivator polypeptide nucleic acid by infection of recipient host cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, polio virus, modified human immunodeficiency virus (HIV), or portions thereof, and the like. See, e.g., U.S. Patent No. 5,624,820, *Episomal Expression Vector for Human Gene Therapy*, issued April 29, 1997.

20 [0154] The KCNQ5 nucleic acid coding region, or desired portion thereof, of the present invention is incorporated into effective eukaryotic expression vectors, which are directly administered or introduced into somatic cells (a nucleic acid fragment comprising a coding region, preferably mRNA transcript, may also be administered directly or introduced into somatic cells). See, e.g., U.S. Patent No. 5,589,466, issued Dec. 31, 1996. Such
30 nucleic acid and vectors may remain episomal, or they may be incorporated into the host chromosomal DNA as a provirus, or portion thereof, that

includes the gene fusion and appropriate eukaryotic transcription and translation signals, i.e., an effectively positioned RNA polymerase promoter 5' to the transcriptional start site and ATG translation initiation codon of the gene fusion, as well as termination codon(s) and transcript polyadenylation signals effectively positioned 3' to the coding region.

[0155] Alternatively, the novel KCNQ5 potassium channel polypeptide DNA can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted DNA transfer using ligand-DNA conjugates or adenovirus-ligand-DNA conjugates, lipofection membrane fusion or direct microinjection, or microparticle bombardment. These procedures and variations thereof are suitable for *ex vivo*, as well as *in vivo* therapies, including use in humans, according to established methods and protocols known in this art.

PCR Diagnostics

[0156] The hKCNQ5 nucleic acid sequence, oligonucleotides, fragments, portions or antisense molecules thereof, may be used in diagnostic assays of body fluids or biopsied tissues to detect the expression level of the KCNQ5 potassium channel molecule in such fluids and tissues. For example, sequences designed from the cDNA sequence SEQ ID NO: 1 or sequences that encode SEQ ID NO:2 (all or a portion thereof) can be used to detect the presence of the mRNA transcripts in a patient or to monitor the modulation of transcripts during treatment.

[0157] One method for amplification of target nucleic acids, or for later analysis by hybridization assays, is the polymerase chain reaction ("PCR") or PCR technique. The PCR technique can be applied to detect sequences of the invention in suspected samples using oligonucleotide primers spaced apart from each other and based on the genetic sequence, e.g., SEQ ID NO: 1, as set forth herein. The primers are complementary to opposite strands of a double stranded DNA molecule and are typically separated by from about 50 to 450 nucleotides or more (usually not more than 2000 nucleotides).

[0158] The PCR method entails preparing specific oligonucleotide primers followed by repeated cycles of target DNA denaturation, primer binding, and extension using a DNA polymerase to obtain DNA fragments of the expected length based on the primer spacing. The present invention provides a diagnostic composition for the identification of a polynucleotide sequence comprising the sequence substantially as depicted in SEQ ID NO:1, or nucleic acid sequences encoding the KCNQ5 polypeptide of SEQ ID NO:2, comprising PCR primers for amplification. The degree of amplification of a target sequence is controlled by the number of cycles that are performed and is theoretically calculated by the simple formula "2n", where n is the number of cycles. See, e.g., Perkin Elmer, *PCR Bibliography*, Roche Molecular Systems, Branchburg, New Jersey; CLONTECH products, Palo Alto, CA; U.S. Patent No. 5,629,158, *Solid Phase Diagnosis of Medical Conditions*, issued May 13, 1997.

15 KCNQ5-Containing Compositions

[0159] Pharmaceutically useful compositions comprising sequences pertaining to the novel hKCNQ5 potassium channel polypeptide, DNA, RNA, antisense sequence, the human KCNQ5 polypeptide itself, or variants and analogs which have biological activity or, otherwise, compounds which modulate KCNQ5 physiology/activity and identified by methods described herein, may be formulated as compositions, preferably physiologically acceptable compositions, according to known methods, such as by the admixture of a pharmaceutically acceptable carrier, diluent, or excipient. Examples of such carriers and methods of formulation may be found in *Remington's Pharmaceutical Sciences*, 18th Ed., 1990, Mack Publishing Co, Easton, PA. To formulate a pharmaceutically acceptable composition suitable for effective administration, preferably *in vivo*, or even *ex vivo*, such compositions will contain an effective amount of the protein, DNA, RNA, or compound modulator.

30 [0160] Therapeutic or diagnostic compositions of the present invention are administered to an individual in amounts effective to treat or diagnose

human physiological disorders, neuroaffective disorders, and/or neurophysiological disorders that are associated with the KCNQ5 potassium channel, its biological activity or physiology. The effective amount may vary according to a variety of factors, such as the individual's condition, weight, sex and age. Other factors include the mode and route of administration. These factors are realized and understood by the skilled practitioner and are routinely taken into account when administering a therapeutic agent to an individual.

[0161] The term "chemical derivative" describes a molecule that contains additional chemical moieties which are not normally a part of the base molecule. Optimally, the chemical moieties do not affect the activity or function of the base molecule, e.g., hKCNQ5. Such moieties may improve the solubility, half-life, absorption, etc. of the base molecule. Alternatively the moieties may attenuate undesirable side effects of the base molecule or decrease the toxicity of the base molecule. Examples of such moieties are described in a number of texts available to the practitioner, such as *Remington's Pharmaceutical Sciences*.

[0162] Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective and sufficient amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art. The therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models, usually mice, rabbits, dogs, pigs, rats, monkeys, or guinea pigs. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. A therapeutically effective dose refers to that amount of the KCNQ5 protein or its antibodies, agonists (openers or activators), or antagonists, (inhibitors or blockers), which ameliorate, reduce or eliminate the symptoms or condition. The exact

dosage is chosen by the individual physician in view of the patient to be treated, the route of administration, the severity of disease, and the like.

[0163] KNCQ5-modulatory compounds identified according to the methods disclosed herein may be used alone, at appropriate dosages

5 defined by routine testing, in order to obtain optimal modulation of a potassium channel biological activity and/or physiological condition, or its activity while minimizing any potential toxicity. In addition, co-administration or sequential administration of other agents may be desirable.

[0164] The pharmaceutical compositions may be provided to an individual
10 in need of therapeutic treatment by a variety of routes, such as subcutaneous, topical, oral, intraperitoneal, intradermal, intravenous, intranasal, rectal, and intramuscular. Administration of pharmaceutical compositions is accomplished orally or parenterally. More specifically, methods of parenteral delivery include topical, intra-arterial (directly to the
15 tissue), intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal administration.

[0165] The present invention also provides suitable topical, oral, systemic and parenteral pharmaceutical formulations for use in the novel methods of treatment comprising the KCNQ5 potassium channel described herein. The
20 compositions containing compounds identified according to described methods to be utilized as the active ingredient in modulating physiological, neurophysiological, or neuropsychological conditions can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compounds can be administered in such
25 oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, the modulatory compounds may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical -- with or without
30 occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. An effective but non-toxic amount

of the compound desired can be employed as a KCNQ5 potassium channel modulating agent.

[0166] The daily dosage of the products may be varied over a wide range, for example, from about 0.01 to 1,000 mg per adult human/per day. For oral administration, the compositions are preferably provided in the form of scored or unscored tablets containing 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, and 50.0 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the individual to be treated. An effective amount of the drug is ordinarily supplied at a dosage level of from about 0.0001 mg/kg to about 100 mg/kg of body weight per day. The range is more particularly from about 0.001 mg/kg to 10 mg/kg of body weight per day. Even more particularly, the range varies from about 0.05 to about 1 mg/kg. Of course, it will be understood by the skilled practitioner that the dosage level will vary depending upon the potency of the particular compound, and that certain compounds will be more potent than others.

[0167] In addition, the dosage level will vary depending upon the bioavailability of the compound. The more bioavailable and potent the compound, the less amount of the compound will need to be administered through any delivery route, including, but not limited to, oral delivery. The dosages of the KCNQ5 potassium channel modulators are adjusted when combined in order to achieve desired effects. On the other hand, dosages of the various agents or modulating compounds may be independently optimized and combined to achieve a synergistic result wherein the pathology is reduced more than it would be if one single agent or compound were used alone. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, the delivery of polynucleotides or polypeptides will be specific to particular cells and conditions.

[0168] In another of its aspects, the present invention provides targeting therapies to deliver an active agent, such as a KCNQ5 protein modulator, or the KCNQ5 polypeptide, antibodies, peptides and nucleic acids of the

present invention, more specifically to certain types of cells, for example, by the use of targeting systems such as antibodies or cell specific ligands.

Targeting may be desirable for a variety of reasons, e.g., if an agent is unacceptably toxic, or if it would require too high a dosage, or if it would not

5 otherwise be able to enter the target cells.

[0169] Rather than administering an active agent directly, the agent could be produced in the target cell, e.g., in a viral vector as described

hereinabove, or in a cell-based delivery system, e.g., as described in U.S.

Patent No. 5,550,050, or published international application numbers WO

10 92/19195, WO 94/25503, WO 95/01203, WO 95/05452, WO 96/02286, WO

96/02646, WO 96/40871, WO 96/40959 and WO 97/12635, designed for

implantation in a patient, i.e., an *ex vivo* type of therapy. The vector can be targeted to the specific cells to be treated, or it can contain regulatory

elements which are more tissue specific to the target cells. The cell-based

15 delivery system is designed to be administered to a patient, or implanted in

a patient's body at the desired target site and contains a coding sequence

for the active agent. Alternatively, the agent can be administered in a

precursor form for conversion to the active form by an activating agent

produced in, or targeted to, the cells to be treated (e.g., see, EP 425 731 A

20 or WO 90/07936).

EXAMPLES

[0170] The following examples as set forth herein are meant to illustrate and exemplify the various aspects of carrying out the present invention and are not intended to limit the invention in any way.

25

EXAMPLE 1

Bioinformatics Analysis

[0171] Human KCNQ2 and KCNQ3 sequences were used as probes to search public domain EST (expressed sequence tag) databases and

proprietary Incyte EST databases. The DNA/protein database search

30 program used was called gapped BLAST2 (Altschul et al., 1997, Nucl. Acids

Res., 25:3389-3402), ("BLAST – basic local alignment search tool"). The outputs from the BLAST search were analyzed for any heretofore unknown channel sequences. By means of the BLAST search, an Incyte #4970006 EST clone was found to correspond to a new channel, and thus was

5 identified from the Incyte database. Bioinformatics analysis revealed homology to a portion of the KCNQ2 gene, thereby suggesting that the clone might be a novel member of KCNQ potassium channel family.

[0172] The Incyte #4970006 clone was fully sequenced and found to contain only a partial gene sequence. Although this clone showed a partial

10 DNA and protein alignment to the KCNQ family (nt 1819-2121), most of the #4970006 clone sequence was hypothesized not to be part of the novel hKCNQ5 clone, based on lack of homology and 5' and 3' RACE experiments. Thus, primers were designed to amplify the short sequence (nt 1819-2121) from the bioinformatic similarity search using human brain

15 tissue as the source nucleic acid. Polymerase chain reaction (PCR) was performed using Human Brain cDNA as a template (Clontech, Marathon-Ready) with primers designed against the region between nt 1819 and nt 2121 of clone #4970006. A "Q5" probe was prepared using the primers: Q5/sense (sense: 5' CTGGATAAGCAGCCACTGTTT 3'), (SEQ ID NO:22)

20 and Q5/antisense (5' GCAGAACATGAGACCACAG 3'), (SEQ ID NO:23). Taq polymerase was used with the following cycling parameters: denaturation -- 94°C for 1 minute, annealing -- 55°C for 1 minute, and extension at 72° C for 1 minute and 30 seconds, for a total of 35 cycles. The DNA product of 259 base pairs (bp) was the expected size and was ligated

25 into the TA vector (pCR 2.1, Invitrogen). Miniprep DNA was analyzed and sequenced.

[0173] The sequencing results indicated that the 259 bp sequence was the same as that of the clone #4970006 between the two priming sites. This PCR product was then used as a probe to screen a human brain cDNA

30 library (Clontech, λ gt10). This 259 bp probe, the Q5 probe (150 ng DNA / 150 μ Ci 32 P-dCTP) was labeled using Ambion's DecaPrime II kit, denatured,

and added to the hybridization solution at $\sim 3\text{-}4 \times 10^6$ cpm/ ml. Specific activity of the probe was $\sim 1\text{-}2 \times 10^9$ cpm/ug.

[0174] For library screening, twenty plates (150 mm) were screened for a total of 10^6 pfu. Plates were lifted using Duralon-UV membranes

5 (Stratagene). The DNA on the membranes was denatured in 1.5 M NaCl, 0.5 M NaOH for 2 minutes, then neutralized in 1.5 M NaCl, 5 M Tris-HCl, pH 8 for 5 minutes, and rinsed in 0.2 M Tris-HCl, pH 7.5, 2X SSC for 5 minutes at room temperature. DNA was UV-crosslinked onto the membranes at 0.5
10 joules. Hybridization of the membranes was overnight at 42° C in 2X PIPES buffer, (i.e., 1,4-piperazine-diethane sulfonic acid), 50% formamide, 1% SDS with 100 µg/ml of denatured salmon testes DNA. Washes were carried out once in 1X SSC, 0.1 % SDS for 15 minutes at room temperature, then 3x in 0.1X SSC, 0.1% SDS for 15-20 minutes each at 55°C. The membranes were exposed to film overnight at -70° C.

15 **[0175]** Following the screening procedure, fifteen initial positive signals were observed, and these plaques were cored from the agar plates, placed in Lambda Dilution Buffer, diluted, and secondary and/or tertiary screening were/was performed with the Q5 probe to isolate the positive plaques. Isolated plaques were cored, placed in dilution buffer, and PCR was
20 performed on the supernatant with λgt10 forward and reverse primers (Forward primer: 5'-CTTTTGAGCAAGTTCAGCCTGGTTAAGT-3' (SEQ ID NO:24); Reverse primer: 5'-GAGGTGGCTTATGAGTATTTCTTCCAGGGTAA-3' (SEQ ID NO:25) to estimate the size of the inserts. DNA was isolated from plate lysates of the
25 positive clones and purified using Promega's Wizard Lambda Prep kit. The DNA was enzymatically digested with *EcoRI*, and inserts were gel-purified using Clontech's Nucleotrap Kit. These inserts were then ligated into the vector pcDNA3.1(+) Neo (Invitrogen).

30 **[0176]** With the full sequence known from the overlapping alignment, the polymerase chain reaction was used to amplify a full length clone from human Brain cDNA (Clontech). To amplify a full length product, the

following primers were designed from the contiguous sequence: [Kozak start 5' GGATATCACCATGAAGGATGTGG 3'], (SEQ ID NO:26) and [Stop primer 5' AATCTAGAACTTATTTTCAGTTTGAC 3'], (SEQ ID NO:27). The following amplification parameters were used: denaturation at 94°C for 30
5 sec, annealing at 55°C for 30 seconds, and extension at 68°C for 3 minutes for a total of 25 cycles. The 2694 bp PCR product was first cloned into pCR2.1 and confirmed by sequence analysis. The hKCNQ5 product was subcloned into pcDNA3.1(+) Neo for expression in mammalian cells and into pBS-KSM for expression in frog oocytes (the pBS-KSM vector has 5' and 3'
10 Xenopus beta globin UTRs for increased expression in Xenopus oocytes).

EXAMPLE 2

RNA expression blots

[0177] To determine the expression of hKCNQ5 mRNA in human tissues, dot blots and Northern blots were performed.

15 **[0178]** For carrying out the dot blot analyses, a Poly A+ RNA Master Blot (Clontech) was hybridized with the same 259 bp Q5 probe used to screen the Human Brain library. Hybridization was done at 65° C overnight in ExpressHyb solution (Clontech) containing 100 ug/ml of denatured salmon testes DNA and denatured Q5 probe (50 ng DNA/ 50 uCi 32-P-dCTP)
20 following the manufacturer's protocol. The blot was washed 5x for 20 minutes each at 65°C in 2X SSC, 0.1% SDS, then 2x for 20 minutes each at 55°C in 0.1X SSC, 0.5% SDS. The blot was exposed to XAR5 film at -70°C.

[0179] In the dot blot shown in Figures 6A and 6B, positive signals were observed in whole brain, as well as for subregions of the brain; including
25 brain subregions including; caudate nucleus, cerebellum, hippocampus, cerebral cortex, frontal lobe, occipital pole, putamen, and temporal lobe. In addition to CNS tissue, skeletal muscle also gave a positive signal. No other tissues appeared to express the KCNQ5 mRNA.

[0180] A Human Multiple Tissue Northern blot (Clontech) was also probed
30 for the presence of hKCNQ5 mRNA. Hybridization was for 1 hour at 68°C in

ExpressHyb solution containing 100 ug/ml of denatured salmon testes DNA and denatured labeled Q5 DNA as probe. The MTN blot was washed for 40 minutes at room temperature in 2x SSC, 0.05% SDS, with continuous agitation and several changes of fresh solution. The blot was then washed
5 3x in 0.1X SSC, 0.1% SDS at 50°C with continuous agitation for 40 min. The blot was exposed overnight to XAR5 film at -70°C. The results are shown in Figures 6A and 6B.

[0181] Similar to the results observed from the dot blot, the human multiple tissue Northern blot revealed a restricted distribution of hKCNQ5
10 mRNA (Figure 7). A strong signal was observed between 7-7.5 Kb for skeletal muscle with a smaller signal in human brain tissue. Although the differences in signal strength could be due to the differences in RNAs between the two blots, the results clearly show a restricted localization to brain tissue and skeletal muscle.

15

EXAMPLE 3

In situ hybridization

[0182] For performing the *in situ* hybridization studies as depicted in Figures 9A/F-18A/B, frozen 16µm sections of rat D0RG, trigeminal, or adult brain cut at intervals of 225 µm were fixed by immersion (without thawing)
20 into ice cold 10% formaldehyde in phosphate buffered saline (PBS) for 20 minutes and rinsed with PBS. Fixed sections were treated with 0.5% Triton X-100 in 0.1 M Tris, pH 8.0, and 0.05 M EDTA for 30 minutes and rinsed for 3 minutes in 0.1 M Tris, pH 8.0, and 0.05 M EDTA. The tissue was then
25 treated with 0.1 M TEA, pH 8.0, plus 0.25% acetic anhydride for 10 minutes at room temperature, rinsed (3 X) in 2X SSC, dehydrated through a series of alcohols, delipidated in chloroform, and air dried.

[0183] Riboprobes were synthesized using the Promega Riboprobe Transcription System II (Promega, LOCATION?) with 250 µCi ³⁵S-UTP and 250 µCi ³⁵S-CTP in a total reaction volume of 10 µL. Unlabeled UTP and
30 CTP were added at 25 µM each and ATP and GTP at 500 µM each. The

human KCNQ5 plasmid (nts 1681-2032 subcloned into pGEM5zf(+)) was linearized with Spe I and transcribed using SP6 RNA polymerase, and with Not I and transcribed using T7 RNA polymerase to generate anti-sense and sense probes, respectively. One µg of linearized plasmid was added for each reaction. The riboprobes were purified by phenol:chloroform extraction and two ethanol precipitations using ammonium acetate. The dried tissue sections were hybridized with 1×10^7 cpm/ml riboprobe in hybridization buffer (50% formamide, 0.3 M NaCl, 10 mM Tris, 1 mM EDTA, 1X Denhardt's solution, 10% dextran sulfate, 500 µg/ml tRNA and 10 mM DTT) overnight at 54 °C. The hybridization solution was removed by rinsing 4 times in 4 x SSC, 5 minutes for each wash. The sections were incubated in 0.02 mg/ml RNase, 0.5 M NaCl, 10 mM Tris, pH 8.0, and 1 mM EDTA for 30 minutes at 37 °C, then washed in 2X SSC, 1X SSC and 0.5X SSC, all containing 1 mM DTT, for 10 minutes per wash at room temperature. The tissues were incubated in 0.1X SSC, 1 mM DTT, and 20% formamide for 30 minutes at 50°C, then rinsed briefly in 0.1X SSC and 1 mM DTT at room temperature, dehydrated, and air dried. The dried sections were exposed to XOMAT film (Kodak, Rochester, NY), then were dipped in NTB2 emulsion (Kodak, Rochester, NY), developed after 2 weeks, and examined under the microscope to determine the cellular localization of each mRNA. Images were acquired using the NIH Image software.

[0184] A summary of the results of the expression of human KCNQ5 mRNA in brain by *in situ* hybridization is presented in Table 2:

Table 2**KCNQ5 *In situ* Hybridization Results**

Region of Brain	Staining Intensity*
Cortex	+
Caudate Putamen	+
Piriform Cortex	++
Septohippocampal nuclei	+
CA2 Region of the Hippocampus	+ / ++
CA3 Region of the Hippocampus	+ / ++
Reticulotegmental Nuclei of the Pons	+ / ++
Pontine Nuclei	+
Dorsal Root Ganglion	+ (in 10-20% of neurons)
Trigeminal Ganglion	+ (in 20-30% of neurons)

*: Intensity of labeling as examined microscopically was scored on a scale of + to ++++++.

5

EXAMPLE 4**hKCNQ5 Oocyte Expression and Electrophysiology**

[0185] Frog (*Xenopus laevis*) oocytes were prepared and injected using standard techniques; each oocyte was injected with approximately 50 nl of hKCNQ5 cRNA. Following injection, oocytes were maintained at 17°C in ND96 medium comprising (in mM): NaCl, 90; KCl, 1.0; CaCl₂, 1.0; MgCl₂, 1.0; HEPES, 5.0; pH 7.5. Horse serum (5%) and penicillin/streptomycin (5%) were added to the incubation medium. Recording commenced 2-6 days following mRNA injection. Prior to the start of an experiment, oocytes were placed in a recording chamber and incubated in Modified Barth's Solution (MBS) consisting of (in mM): NaCl, 88; NaHCO₃, 2.4; KCl, 1.0;

15

HEPES, 10; MgSO_4 , 0.82; $\text{Ca}(\text{NO}_3)_2$, 0.33; CaCl_2 , 0.41; pH 7.5. Oocytes were impaled with electrodes (1-2 $\text{M}\Omega$) and standard 2-electrode voltage clamp techniques were employed to record whole-cell membrane currents. Voltage-clamp protocols typically consisted of a series of voltage steps 1-5 second duration, in +10 mV steps from a holding potential of -90 mV to a maximal potential of +40 mV; records were digitized at 5 kHz and stored on a computer using pClamp data acquisition and analysis software (Axon Instruments).

[0186] As demonstrated in Figure 8, the injection of hKCNQ5 cRNA into *Xenopus* oocytes resulted in robust expression of large outward currents under the recording conditions employed in these experiments. Currents appeared similar to those obtained by the expression of KCNQ2 channels; currents were slowly activating, non-inactivating at positive voltage steps over a period of seconds, and deactivated with a time course similar to the current activation. However, unlike the KCNQ2 currents, the hKCNQ5 currents were largely resistant to the potassium channel blocker tetraethylammonium (TEA), even at concentrations as high as 30 mM.

[0187] These results indicated that expression of the hKCNQ5 construct in oocytes produced robust expression of large outward currents similar to those seen with KCNQ2 expression, but with a different pharmacology. Injection of equivalent amounts of water (50 nl) did not result in expression of outward currents at the voltage steps used to detect KCNQ5 expression.

EXAMPLE 5

Ligand binding assay for high throughput screening for hKCNQ5 modulators

[0188] Cell lines that over-express the heterologous KCNQ5 potassium channel coding region described herein (SEQ ID NO:1), or a biologically active fragment or truncated portion thereof, or a chimeric or fusion protein, are used in binding assays to identify and screen for pharmacologically active molecules that block hKCNQ5 activity.

[0189] A radiolabeled binding assay using a radiolabeled ligand is employed (Hill, R.J., 1995, Mol. Pharm., 48:98 and Deutsch, C. et al., 1991, J. Biol. Chem., 266:3668). Membrane preparations of cell lines that over-express the KCNQ5 potassium channel are made by homogenizing the cells using a Polytron for 25 seconds at 13,000 rpm and centrifuged at 100xg for 2 minutes. The pellet is suspended in 1 ml of assay buffer (5 mM NaCl, 5 mM KCl, 10 mM HEPES, 6mM glucose, pH 8.4) and diluted to 50 µg/ml.

[0190] To each of the wells of a 96-well microtiter plate, 130 µl of assay buffer is added, along with 20 µl of test compound or drug (the test compound or drug may be a small molecule, peptide, analog, or mimetic agent), control assay buffer, non-specific, unlabeled ligand (10 nM), 50 µl of membranes from cells over-expressing KCNQ5 potassium channel at 50 µg/ml, and 50 µl of radioligand (25 pM; NEN, 2200 Ci/mmol). The plates are incubated for 20 minutes at 21°C with mixing. Bound radiolabeled ligand is separated from free radiolabeled ligand in solution by filtration over pre-soaked GF/C Unifilters (Packard Instruments) and washed rapidly in ice-cold wash buffer. Upon drying, scintillation fluid is added and the filter plates are scintillation counted. Data from saturation experiments are subjected to Scatchard analysis and linear regression. (Deutsch, C. et al., 1991, J. Biol. Chem., 266:3668). Compounds that compete with the radiolabeled ligand for binding the novel KCNQ5 potassium channel are identified via the production of a reduction in specific counts. Alternatively, a scintillation proximity assay (SPA) can be used so as to eliminate the need for filters. SPA is easily adapted for high throughput screening assays (Hoffman, R. et al., 1992, Anal. Biochem., 29:370; Kienuis, C.B.M. et al., 1992, J. Recept. Res., 12:389).

[0191] The contents of all patents, patent applications, published articles, books, reference manuals and abstracts cited herein are hereby incorporated by reference in their entirety to more fully describe the state of the art to which the invention pertains.

[0192] As various changes can be made in the above-described subject matter without departing from the scope and spirit of the present invention, it is intended that all subject matter contained in the above description, or defined in the appended claims, be interpreted as descriptive and illustrative of the present invention. Many modifications and variations of the present invention are possible in light of the above teachings.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide molecule encoding a polypeptide comprising all or a portion of a human KCNQ5 protein.
2. The polynucleotide molecule according to claim 1, wherein the
5 human KCNQ5 protein comprises the amino acid sequence set forth in SEQ ID NO:2.
3. The polynucleotide molecule according to claim 1, wherein the molecule is selected from the group consisting of (a) all or a portion of a nucleic acid sequence set forth in SEQ ID NO:1; (b) the complement of (a);
10 and (c) variations of (a) due to degeneracy in the genetic code.
4. A vector comprising the polynucleotide molecule according to claim 1.
5. A vector comprising the polynucleotide molecule according to claim 2.
- 15 6. A vector comprising the polynucleotide molecule according to claim 3.
7. A host cell comprising the vector according to claim 4.
8. A host cell comprising the vector according to claim 5.
9. A host cell comprising the vector according to claim 6.
- 20 10. The host cell according to claim 7, wherein said cell is prokaryotic or eukaryotic.
11. The host cell according to claim 8, wherein said cell is prokaryotic or eukaryotic.
12. The host cell according to claim 9, wherein said cell is
25 prokaryotic or eukaryotic.
13. An isolated nucleic acid molecule having at least 80% sequence identity to the nucleotide sequence as shown in SEQ ID NO:1.
14. An isolated nucleic acid molecule encoding a human KCNQ5 potassium channel, wherein said nucleic acid molecule hybridizes under
30 moderate stringency conditions to a nucleic acid encoding the amino acid

sequence set forth in SEQ ID NO:2.

15. An isolated nucleic acid molecule encoding a human KCNQ5 potassium channel polypeptide, wherein said nucleic acid molecule hybridizes under high stringency conditions to a nucleic acid encoding the amino acid sequence set forth in SEQ ID NO:2.

16. An isolated nucleic acid molecule encoding a human KCNQ5 potassium channel polypeptide and having a contiguous nucleotide sequence that encodes the amino acid sequence set forth in SEQ ID NO:2.

17. A vector comprising the nucleic acid molecule according to claim 13.

18. A vector comprising the nucleic acid molecule according to claim 14.

19. A vector comprising the nucleic acid molecule according to claim 15.

20. A vector comprising the nucleic acid molecule according to claim 16.

21. A cell comprising the vector according to claim 17.

22. A cell comprising the vector according to claim 18.

23. A cell comprising the vector according to claim 19.

24. A cell comprising the vector according to claim 20.

25. An isolated KCNQ5 polypeptide comprising all or a portion of the amino acid sequence set forth in SEQ ID NO:2.

26. An isolated KCNQ5 polypeptide encoded by the polynucleotide molecule according to any of claims 1 to 3.

27. An isolated KCNQ5 polypeptide encoded by the nucleic acid molecule according to any of claims 13 to 16.

28. A method of identifying a compound that modulates the biological activity of a KCNQ5 potassium channel polypeptide, comprising:

- (a) combining a candidate modulator compound with a KCNQ5 potassium channel polypeptide having the sequence set forth in SEQ ID NO:2; and

(b) measuring an effect of the candidate modulator compound on the activity of the KCNQ5 potassium channel polypeptide.

29. A method of identifying a compound that modulates the biological activity of a KCNQ5 potassium channel polypeptide, comprising:

5 (a) combining a candidate modulator compound with a host cell expressing a KCNQ5 potassium channel polypeptide having the sequence as set forth in SEQ ID NO:2; and

(b) measuring an effect of the candidate modulator compound on the activity of the expressed KCNQ5 potassium channel polypeptide.

10 30. A method of identifying a compound that modulates the biological activity of a KCNQ5 potassium channel polypeptide, comprising:

(a) combining a candidate modulator compound with a host cell containing a vector according to any of claims 4 to 6, or claims 17 to 20, wherein a KCNQ5 potassium channel polypeptide is expressed by the cell; and

(b) measuring an effect of the candidate modulator compound on the activity of the expressed KCNQ5 potassium channel polypeptide.

20 31. A method of screening for a compound that is capable of modulating the biological activity of a KCNQ5 polypeptide, comprising the steps of:

(a) providing a host cell according to any of claims 7 to 9 and 21 to 24;

(b) determining the biological activity of the KCNQ5 polypeptide in the absence of a modulator compound;

25 (c) contacting the cell with the modulator compound; and

(d) determining the biological activity of the KCNQ5 polypeptide in the presence of the modulator compound; wherein a difference between the activity of the KCNQ5 polypeptide in the presence of the modulator compound and in the absence of the modulator compound indicates a modulating effect of the compound.

30 32. A compound that modulates the biological activity of human

KCNQ5 potassium channel polypeptide as identified by the method according to claim 28.

33. A compound that modulates the biological activity of human KCNQ5 potassium channel polypeptide as identified by the method
5 according to claim 29.

34. A compound that modulates the biological activity of human KCNQ5 potassium channel polypeptide as identified by the method according to claim 30.

35. A compound that modulates the biological activity of human
10 KCNQ5 potassium channel polypeptide as identified by the method according to claim 31.

36. An antisense nucleic acid molecule which binds to the polynucleotide molecule according to claim 1.

37. An antisense nucleic acid molecule which binds to the
15 polynucleotide molecule according to claim 2.

38. An antisense nucleic acid molecule which binds to the polynucleotide molecule according to claim 3.

39. An antisense nucleic acid molecule which binds to the nucleic acid molecule according to any of claims 13 to 16.

20 40. A pharmaceutical composition comprising the polypeptide according to claim 25 and a physiologically acceptable carrier, excipient, or diluent.

41. A pharmaceutical composition comprising the polypeptide according to claim 26 and a physiologically acceptable carrier, excipient, or
25 diluent.

42. A pharmaceutical composition comprising the polypeptide according to claim 27 and a physiologically acceptable carrier, excipient, or diluent.

43. A method for the production of a pharmaceutical composition
30 comprising the steps of the method according to claim 28 and further including step (c): formulating the compound that is capable of modulating

the biological activity of the KCNQ5 polypeptide in a pharmaceutically acceptable form.

44. A method for the production of a pharmaceutical composition comprising the steps of the method according to claim 29 and further
5 including step (c): formulating the compound that is capable of modulating the biological activity of the KCNQ5 polypeptide in a pharmaceutically acceptable form.

45. A method for the production of a pharmaceutical composition comprising the steps of the method according to claim 30 and further
10 including step (c): formulating the compound that is capable of modulating the biological activity of the KCNQ5 polypeptide in a pharmaceutically acceptable form.

46. A method for the production of a pharmaceutical composition comprising the steps of the method according to claim 31 and further
15 including step (e): formulating the compound that is capable of modulating the biological activity of the KCNQ5 polypeptide in a pharmaceutically acceptable form.

47. The method according to claim 43, wherein the pharmaceutical composition is for the treatment of acute and chronic pain, migraine
20 headache, acute stroke, dementia, trauma, epilepsy, seizure, amyelotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Parkinson's Disease, anxiety disorders, depression, bipolar disorders, sleep disorders, addiction, eating disorders, ataxia, myokymia, Alzheimer's disease, age-associated memory loss, learning deficiencies, cognitive disorders, and motor neuron diseases.

25 48. The method according to claim 44, wherein the pharmaceutical composition is for the treatment of acute and chronic pain, migraine headache, acute stroke, dementia, trauma, epilepsy, seizure, amyelotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Parkinson's Disease, anxiety disorders, depression, bipolar disorders, sleep disorders, addiction, eating
30 disorders, ataxia, myokymia, Alzheimer's disease, age-associated memory loss, learning deficiencies, cognitive disorders, and motor neuron diseases.

49. The method according to claim 45, wherein the pharmaceutical composition is for the treatment of acute and chronic pain, migraine headache, acute stroke, dementia, trauma, epilepsy, seizure, amyelotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Parkinson's Disease, anxiety disorders, depression, bipolar disorders, sleep disorders, addiction, eating disorders, ataxia, myokymia, Alzheimer's disease, age-associated memory loss, learning deficiencies, cognitive disorders, and motor neuron diseases.

50. The method according to claim 46, wherein the pharmaceutical composition is for the treatment of acute and chronic pain, migraine headache, acute stroke, dementia, trauma, epilepsy, seizure, amyelotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Parkinson's Disease, anxiety disorders, depression, bipolar disorders, sleep disorders, addiction, eating disorders, ataxia, myokymia, Alzheimer's disease, age-associated memory loss, learning deficiencies, and motor neuron diseases.

51. An antibody specific for the KCNQ5 polypeptide according to claim 25.

52. An antibody specific for the KCNQ5 polypeptide according to claim 26.

53. An antibody specific for the KCNQ5 polypeptide according to claim 27.

54. The antibody according to claim 51, wherein the antibody is a monoclonal antibody.

55. The antibody according to claim 52, wherein the antibody is a monoclonal antibody.

56. The antibody according to claim 53, wherein the antibody is a monoclonal antibody.

57. The antibody according to claim 51 or claim 54, wherein said antibody is directed toward a portion of the KCNQ5 polypeptide comprising a peptide having a sequence selected from the group consisting of SEQ ID NO:5-21, or a combination thereof.

58. An isolated polynucleotide molecule selected from the group

consisting of an allelic variant, an alternative splice exon variant, and a chimeric fusion channel of the polypeptide encoded by the polynucleotide according to any of claims 1 to 3.

59. An isolated polynucleotide molecule selected from the group
5 consisting of an allelic variant, an alternative splice exon variant, and a chimeric fusion channel of the polypeptide encoded by the nucleic acid molecule according to any of claims 13 to 16.

60. A vector according to any of claims 4-6 or 17-20, selected from the group consisting of viral, prokaryotic and eukaryotic vectors.

10 61. A method for screening a plurality of compounds to assess specific binding affinity with a KCNQ5 potassium channel polypeptide, or a bindable portion thereof, comprising:

(a) providing a plurality of test compounds;
(b) combining the KCNQ5 polypeptide, or a bindable fragment
15 thereof, with each of the plurality of compounds for a time sufficient to allow binding under suitable conditions; and
(c) detecting binding of the KCNQ5 polypeptide to each of the plurality of test compounds, thereby identifying the compounds that specifically bind to the KCNQ5 polypeptide.

20 62. An isolated nucleic acid molecule, wherein the sequence of said nucleic acid molecule is identical to the sequence in ATCC Deposit No. PTA-1924 (human KCNQ5).

63. A method of treating a disease or disorder selected from the group consisting of acute and chronic pain, migraine headaches, acute
25 stroke, dementia, vascular dementia, trauma, epilepsy, seizures, asthma, amyelotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Parkinson's Disease, neurophysiological disorders, neuropsychological disorders, anxiety disorders, depression, bipolar disorders, sleep disorders, addiction, eating disorders, ataxia, myokymia, Alzheimer's disease, age-associated
30 memory loss, cognitive disorders, learning deficiencies, neuronal cell death, and brain tumors and motor neuron diseases, comprising providing the

KCNQ nucleic acid molecule of claim 1, claim 2, or claim 3 in an amount effective to treat the disease or disorder in a physiologically acceptable carrier or excipient.

64. A method of screening for or detecting candidate compounds
5 capable of binding to human KCNQ5 potassium channel polypeptide,
comprising:

- a) contacting a test compound with a purified KCNQ5
polypeptide according to claim 25; and
- b) selecting as candidate compounds those test
10 compounds that bind to the polypeptide.

65. A method of screening for or detecting candidate compounds
capable of binding to human KCNQ5 potassium channel polypeptide,
comprising:

- a) contacting a test compound with a purified KCNQ5
15 polypeptide according to claim 26; and
- b) selecting as candidate compounds those test
compounds that bind to the polypeptide.

66. A method of screening for or detecting candidate compounds
capable of binding to human KCNQ5 potassium channel polypeptide,
20 comprising:

- a) contacting a test compound with a purified KCNQ5
polypeptide according to claim 27; and
- b) selecting as candidate compounds those test
compounds that bind to the polypeptide.

25 67. The method according to claim 64, wherein the polypeptide is
immobilized onto a solid support.

68. The method according to claim 65, wherein the polypeptide is
immobilized onto a solid support.

69. The method according to claim 66, wherein the polypeptide is
30 immobilized onto a solid support.

70. The method according to claim 64, wherein the candidate compounds are immobilized onto a support.

71. The method according to claim 65, wherein the candidate compounds are immobilized onto a support.

5 72. The method according to claim 66, wherein the candidate compounds are immobilized onto a support.

73. The method according to claim 64, wherein the method comprises high throughput screening technology.

10 74. The method according to claim 65, wherein the method comprises high throughput screening technology.

75. The method according to claim 66, wherein the method comprises high throughput screening technology.

76. The method according to claim 64, wherein the candidate compounds are small molecules, therapeutics, or drugs.

15 77. The method according to claim 65, wherein the candidate compounds are small molecules, therapeutics, or drugs.

78. The method according to claim 66, wherein the candidate compounds are small molecules, therapeutics, or drugs.

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FIG. 1A

KCNQ5 cDNA coding sequence

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atgaaggatg tggagtcggg ccggggcagg gtgctgctga 40
actcggcagc cgccaggggc gacggcctgc tactgctggg 80
caccgcgcgc gccacgcttg gtggcggcgg cggcggcctg 120
agggagagcc gccggggcaa gcagggggcc cggatgagcc 160
tgctggggaa gccgctctct tacacgagta gccagagctg 200
ccggcgcaac gtcaagtacc ggcggtgca gaactacctg 240
tacaacgtgc tggagagacc ccgcggctgg gcgttcatct 280
accacgcttt cgtttttctc cttgtctttg gttgcttgat 320
tttgtcagtg ttttctacca tcctgagca cacaaaattg 360
gcctcaagtt gcctcttgat cctggagttc gtgatgattg 400
tcgtctttgg tttggagttc atcattcgaa tctggtctgc 440
gggttgctgt tgctgatata gaggatggca aggaagactg 480
aggtttgctc gaaagccctt ctgtgttata gataccattg 520
ttcttatcgc ttcaatagca gttgtttctg caaaaactca 560
gggtaatat tttgccacgt ctgcactcag aagtctccgt 600
ttcctacaga tcctccgcat ggtgcgcatg gaccgaagg 640
gaggcacttg gaaattactg gggtcagtgg tttatgctca 680
cagcaaggaa ttaatcacag cttggtacat aggatttttg 720
gttcttatatt tttcgtcttt cttgtctat ctggtggaaa 760
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FIG. 1B

aggatgccaa taaagagttt tctacatatg cagatgctct 800
ctggtggggc acaattacat tgacaactat tggctatgga 840
gacaaaactc ccctaacttg gctgggaaga ttgctttctg 880
caggctttgc actccttggc atttctttct ttgcacttcc 920
tgccggcatt cttggctcag gttttgcatt aaaagtacaa 960
gaacaacacc gccagaaaca ctttgagaaa agaaggaacc 1000
cagctgccaa cctcattcag tgtgtttggc gtagttacgc 1040
agctgatgag aaatctgttt ccattgcaac ctggaagcca 1080
cacttgaagg ccttgcacac ctgcagccct accaagaaag 1120
aacaagggga agcatcaagc agtcagaagc taagttttaa 1160
ggagcgagtg cgcattggcta gcccagggg ccagagtatt 1200
aagagccgac aagcctcagt aggtgacagg aggtcccaa 1240
gcaccgacat cacagccgag ggcagtccca ccaaagtgca 1280
gaagagctgg agcttcaacg accgaaccg cttccggccc 1320
tcgctgcgcc tcaaaagttc tcagccaaaa ccagtgatag 1360
atgctgacac agcccttggc actgatgatg tatatgatga 1400
aaaaggatgc cagtgtgatg tatcagtgga agacctcacc 1440
ccaccactta aaactgtcat tcgagctatc agaattatga 1480
aatattcatgt tgcaaaacgg aagtttaagg aaacgttacg 1520
tccatatgat gtaaaagatg tcattgaaca atattctgct 1560

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FIG. 1C

ggtcacatctgg acatgtttgtg tagaattaaa agccttcaaa 1600
cacgtgttga tcaaattctt ggaaaagggc aaatcacatc 1640
agataagaag agccgagaga aaataacagc agaacatgag 1680
accacagacg atctcagtat gctcggtcgg gtggtcaagg 1720
ttgaaaaaca ggtacagtcc atagagtcca agctggactg 1760
cctactagac atctatcaac aggtccttcg gaaaggctct 1800
gcctcagccc tcgctttggc ttcattccag atcccacctt 1840
ttgaatgtga acagacatct gactatcaaa gccctgtgga 1880
tagcaaagat ctttcggggt cgcacaaaa cagtggctgc 1920
ttatccagat caactagtgc caacatctcg agaggcctgc 1960
agttcattct gacgcctaat gagttcagtg cccagacttt 2000
ctacgcgctt agccctacta tgcacagtca agcaacacag 2040
gtgccaatta gtcaaagcga tggctcagca gtggcagcca 2080
ccaacaccat tgcaaacc aaataacagg cacccaagcc 2120
agcagcccca acaactttac agatcccacc tcctctccca 2160
gccatcaagc atctgcccag gccagaaact ctgcacccta 2200
accctgcagg cttacaggaa agcatttctg acgtcaccac 2240
ctgccttggt gcctccaagg aaaatgttca ggttgcacag 2280
tcaaattctca ccaaggaccg ttctatgagg aaaagctttg 2320
acatgggagg agaaactctg ttgtctgtct gtcccatggt 2360
gccgaaggac ttgggcaaat ctttgtctgt gcaaaacctg 2400
atcaggtcga ccgaggaact gaatatacaa ctttcaggga 2440

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FIG. 1D

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gtgagtcaag tggctccaga ggcagccaag atttttaccc 2480
caaatggagg gaatccaaat tgtttataac tgatgaagag 2520
gtgggtcccg aagagacaga gacagacact tttgatgccg 2560
caccgcagcc tgccaggga gctgcctttg catcagactc 2600
tctaaggact ggaaggtcac gatcatctca gagcatttgt 2640
aaggcaggag aaagtacaga tgccttcagc ttgcctcatg 2680
tcaaactgaa ataa 2694
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FIG. 2A

KCNQ5 Protein Sequence

MKDVESGRGR	VLLNSAAARG	DGLLLLGTRA	ATLGGGGGGL	40
RESRRGKQGA	RMSLLGKPLS	YTSSQSCRRN	VKYRRVQNYL	80
YNVLERPRGW	AFIYHAFVFL	LVFGCLILSV	FSTIPEHTKL	120
ASSCLLILEF	VMIVVFGLEF	IIRIWSAGCC	CRYRGWQGRL	160
RFARKPFCVI	DTIVLIASIA	VVSAKTQGNI	FATSALRSLR	200
FLQILRMVRM	DRRGGTWKL	GSVVYAHSKE	LITAWYIGFL	240
VLIFSSFLVY	LVEKDANKEF	STYADALWWG	TITLTTIGYG	280
DKTPLTWLGR	LLSAGFALLG	ISFFALPAGI	LGSGFALKVQ	320
EQHRQKHFEK	RRNPAANLIQ	CVWRSYAADE	KSVSIATWKP	360
HLKALHTCSP	TKKEQGEASS	SQKLSFKERV	RMASPRGQSI	400
KSRQASVGDR	RSPSTDITAE	GSPTKVQKSW	SFNDRTFRFP	440
SLRLKSSQPK	PVIDADTALG	TDDVYDEKGC	QCDVSVEDLT	480
PPLKTVIRAI	RIMKFHVAKR	KFKETLRPYD	VKDVIEQYSA	520
GHLDMLCRIK	SLQTRVDQIL	GKGQITSDDK	SREKITAEHE	560
TTDDL SMLGR	VVKVEKQVQS	IESKLDCLLD	IYQQVLRKGS	600
ASALALASFQ	IPPFCEQTS	DYQSPVDSKD	LSGSAQNSGC	640
LSRSTSANIS	RGLQFILTPN	EFSAQTFYAL	SPTMHSQATQ	680
VPISQSDGSA	VAATNTIANQ	INTAPKPAAP	TTLQIPPLP	720
AIKHLPRPET	LHPNPAGLQE	SISDVTTCLV	ASKENVQVAQ	760
SNLTKDRSMR	KSFDMGGETL	LSVCPMVPKD	LGKSLSVQNL	800
IRSTEELNIQ	LSGSESSGSR	GSQDFYPKWR	ESKLFITDEE	840

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FIG. 2B

VGPEETETDT FDAAPQPARE AAFASDSLRT GRSRSSQSIC	880
KAGESTDALS LPHVKLK	897

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FIG. 3**Alternative Splice Exon 1**

TGG	GGA	CAG	TGG	ACA	TTG	CGT
Trp	Gly	Gln	Trp	Thr	Leu	Arg

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FIG. 4A

3' UTR

gttccttcatt ttctttccag gcatagcagt tcttttagcca	40
tacatatcat tgcatgaact atttcgaaag cccttctaaa	80
aagttgaaat tgcaagaatc gggaagaaca tgaaaggcag	120
tttataagcc cgttaccttt taattgcatg aaaatgcatg	160
tttagggatg gctaaaattc caaggtgcat cgacattaac	200
ccactcattt agtaatgtac cttgaggtta aaagcctgag	240
aaaccaaaca cagctaattgc tatgggggtgt atgaatatgt	280
caagtttagg tcatttagaa gatttgacac tgtattttga	320
aattatgagt aaacaccttc aaatttcagg catttctgct	360
ttgtgactaa atacaaacta cattttcaag attaggccat	400
aatgtatatt taaacacaat ggctatcaac agctgctaata	440
aaggatatcaa ctaaagcaga attggggaat aatagaaatg	480
gctgcttatt tcaagatata ttgccaacc cattcctatt	520
cagtcatttt attattaatg taatttgaat gtcaatttgt	560
gtgcttttgg tgatttagcg ctgtggcaag caattttgca	600
catcattttc atgttggttct ttatgacaag aatgttcttc	640
aattagaaaa tgtgcaaata atgaaattca gggccagtga	680
ggcaaataga ctatctgaca tatttgactt tatgaaaaca	720
tattgcctga tggcagaatc aactttataa gtggtcaact	760
tctacacaag cgtatgaaat actggtcagt agaacagcca	800

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FIG. 4B

ttgtgattgg actggtttct ctgcaatggc gccaacccca	840
ggcttgccaa tactgcctat gtaaagggca agtgtgagaa	880
gctatttctca tttcgctgac atacaggtag gactatgggg	920
gatgggacat ttgagtggga ctgagatagg aaaggcttga	960
aaagaaccca gaaacaccac caggaagttg gcaaagtaaa	1000
agaaaatgac ttccccctca aagggaatg agaggagag	1040
aaacaaacca aaatagaaga actagacttt ttagaaaatg	1080
agtattgcta	1090

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FIG. 5A

Multiple Sequence Alignment of the KCNQ Channel Family Members

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hskcnq4 ~~~~~~MAEAPPRRLGLGPPPGDAPRAE.LVALTAVQSEOGEGAG.
hskcnq5 ~~~~~~MKDVESGR.GRVLLNSAAARGDGLLLLGTAAATLGCGG.
hskcnq2 ~~~~~~MVQKSRNG.GVYPG..PSGEKKLVGVFVGLDPG.AP.
hskcnq3 MGLKARRAAGAAGGGGDDGGGGGGAANPAGCDAAAAGDEERKVGLAPGDVEQVTIALGA.
hskcnq1 ~~~~~~MAAASSPPRAERKRWGWRGLPGARRGSAGLAKKCPFSLELAEGGP

hskcnq4 GGGSPRRRLGLLGSPLP.PGAPLPGPGSG.SGSACGORSSAA.HKR...Y..RR.....
hskcnq5 GGLRESRRGKQGARMSSLLGKPL...SYT.SSQSC.RR.....NVK...Y..RR.....
hskcnq2 DSTRDGALLIAGSEAPKRGSI LSKPRAG.GAGA..GKPPKR.NAF...Y..RK.....
hskcnq3 GADKDGTLLLEGGGRDE.GQRRTPOGIGLLAKTPLSRPVKRNNAK...Y..RR.....
hskcnq1 AGGALYAPIAPGAPGPAPPASPAAPAAPPVASDLGPPPVSLDPRVSIYSTRPVLARTH

hskcnq4 LONWYINVLERPRGW.AFVYHVFTFLVFSCLVLSVLSSTIQEHQELANECLLIEFVMTIV
hskcnq5 VONYEINVLERPRGW.AFIYHAFVFLVFGCLVLSVFSSTIPEHTKLASSCLLIEFVMTIV
hskcnq2 LONFLYINVLERPRGW.AFIYHAYVFLVFSCLVLSVFSSTIKEYEKSSEGALEYIEIVTIV
hskcnq3 TOTLYDALERPRGW.ALLYHALVFLVFLGCLVLAFLTFKEYETVSGDWLLLETFAEF
hskcnq1 VOGRVYNFLERPTGWKCFVYHFVFLVFLVCLVFSVLSSTIEQYAALATGTFWMEIVLVV

hskcnq4 VFGLEYIVRVWSAGCCCRYRGWQGRFRFARKEFCVIDFIVFVASMAVLAAGTOGNIFATS
hskcnq5 VFGLEYITRIWSAGCCCRYRGWQGRRLRFARKEFCVIDTIVLTASTAVVSAKTOGNIFATS
hskcnq2 VFGVEYFVRIWAAGCCCRYRGWRGRLKFARKEFCVIDIMVLTAATAVLAAGSQGNVIFATS
hskcnq3 IFGAEEALRIWAAGCCCRYKGWRGRLKFARKPLCMEDIFVLTAAPVVAAGNQGNVIFATS
hskcnq1 FFGTEYVVRILWSAGCRSKYVGLWGRRLRFARKEISTIDLIIVVASMVVLVCGSKGOVFATS

hskcnq4 ALRSLRFLQILRMVRMDRRGGTWKLLGSVMYAHSKELITAWYIGFLVLIFASFLVYLAEK
hskcnq5 ALRSLRFLQILRMVRMDRRGGTWKLLGSVMYAHSKELITAWYIGFLVLIFSSFLVYLVEK
hskcnq2 ALRSLRFLQILRMVRMDRRGGTWKLLGSVMYAHSKELITAWYIGFLCLILASFLVYLAEK
hskcnq3 .LRSLRFLQILRMVRMDRRGGTWKLLGSATCAHSKELITAWYIGFLTILSSFLVYLVEK
hskcnq1 ALRGLRFLQILRMVRMDRQGGTWKLLGSVMFIHQELITITLYIGFLGLIFSSFLVYLAEK

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hskcnq3 VVSFPFFR.....KEQLEAA.....
hskcnq1 .....IRKAP..RSHTLLS.....PSPKPKSVVVKKKKFKLDDKNGVT

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FIG. 5B

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FIG. 5C

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hskcnq1 ~~~~~~

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FIG. 6A

Human RNA Master Blot

TABLE 1

	1	2	3	4	5	6	7	8
A	whole brain	amygdala	Caudate nucleus	cerebellum	cerebral cortex	frontal lobe	hippocampus	medulla oblongata
B	occipital pole	putamen	substantia nigra	temporal lobe	thalamus	Subthalamic nucleus	spinal cord	
C	heart	aorta	Skeletal muscle	colon	bladder	uterus	prostate	stomach
D	testis	ovary	pancreas	pituitary gland	adrenal gland	thyroid gland	salivary gland	mammary gland
E	kidney	liver	small intestine	spleen	thymus	peripheral leukocyte	lymph node	bone marrow
F	Appendix	lung	trachea	placenta				
G	fetal brain	fetal heart	fetal kidney	fetal liver	fetal spleen	fetal thymus	fetal lung	
H	yeast total RNA	yeast tRNA	<i>E. coli</i> rRNA	<i>E. coli</i> DNA	Poly r(A)	human C ₀ t DNA	human DNA	human DNA

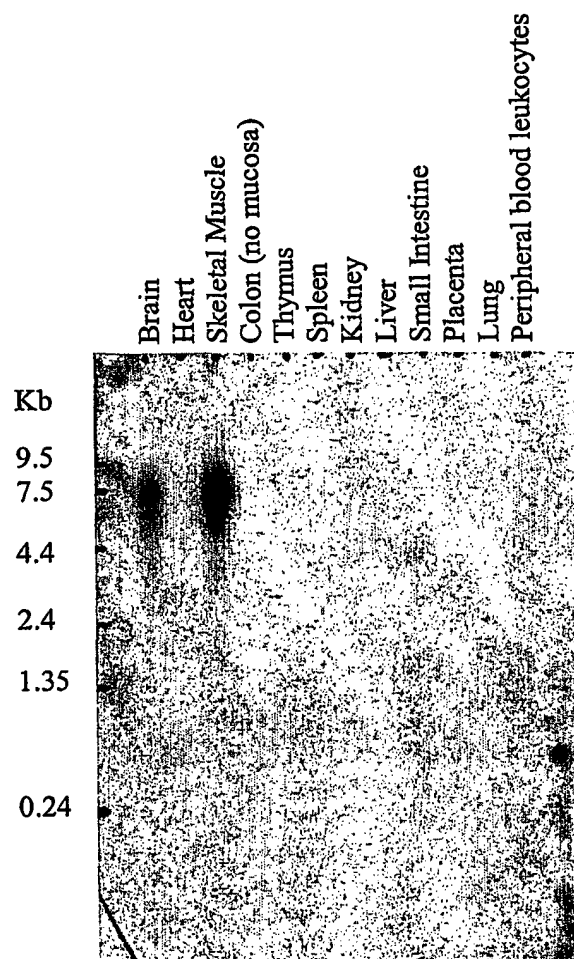
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FIG. 6B

	1	2	3	4	5	6	7	8
A	•		•		•	•	•	
B	•	•		•				
C			•					
D								
E								
F								
G								
H				•				

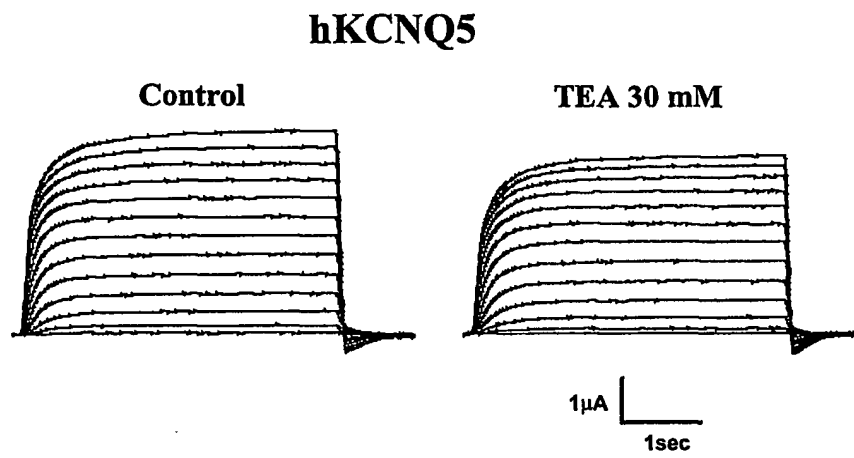
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FIG. 7



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FIG. 8



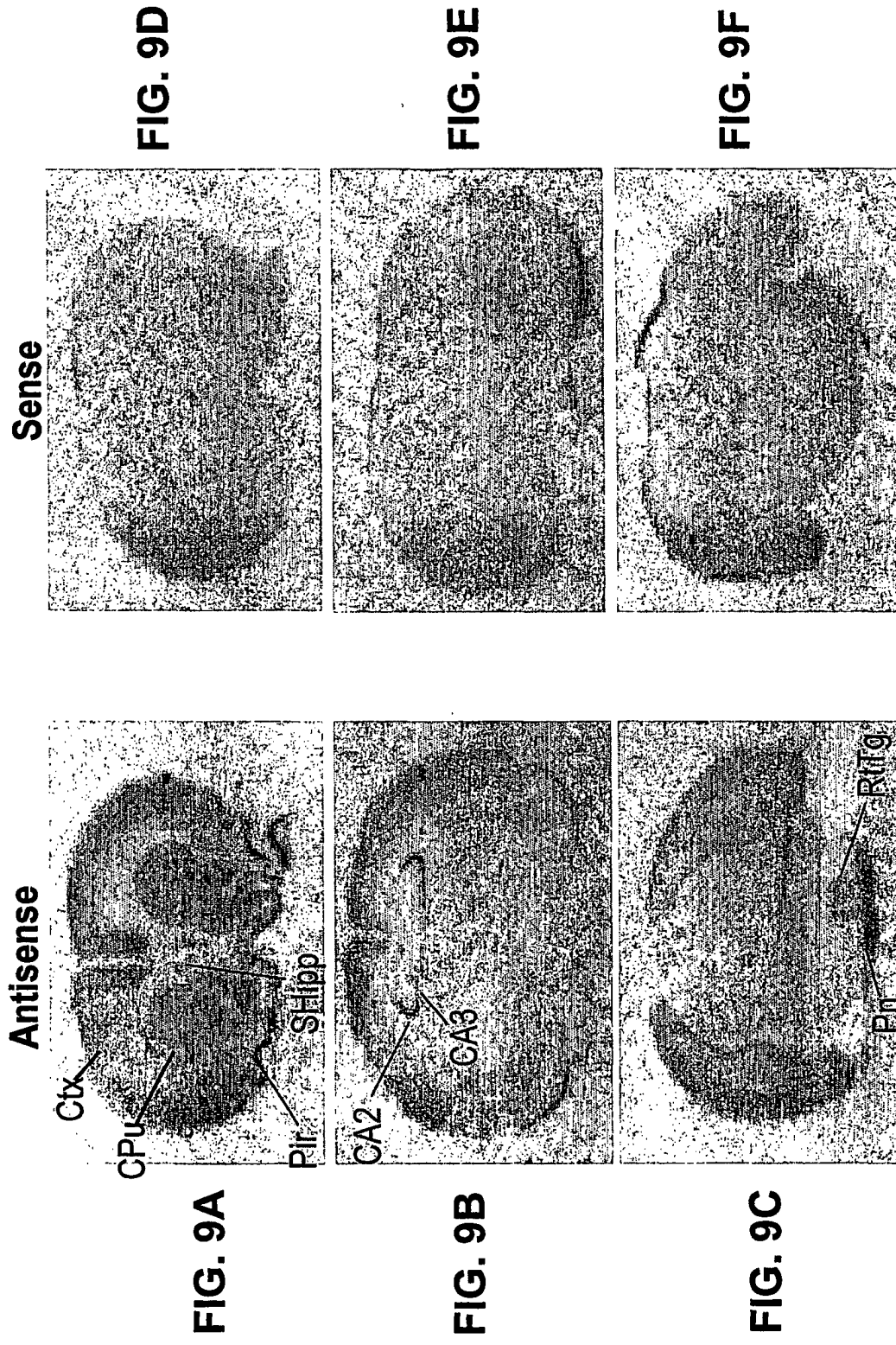


FIG. 10B

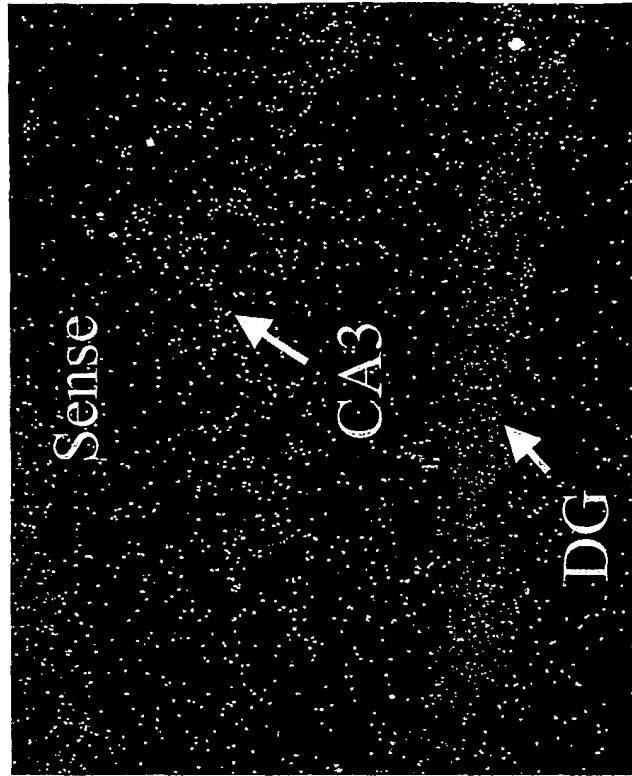
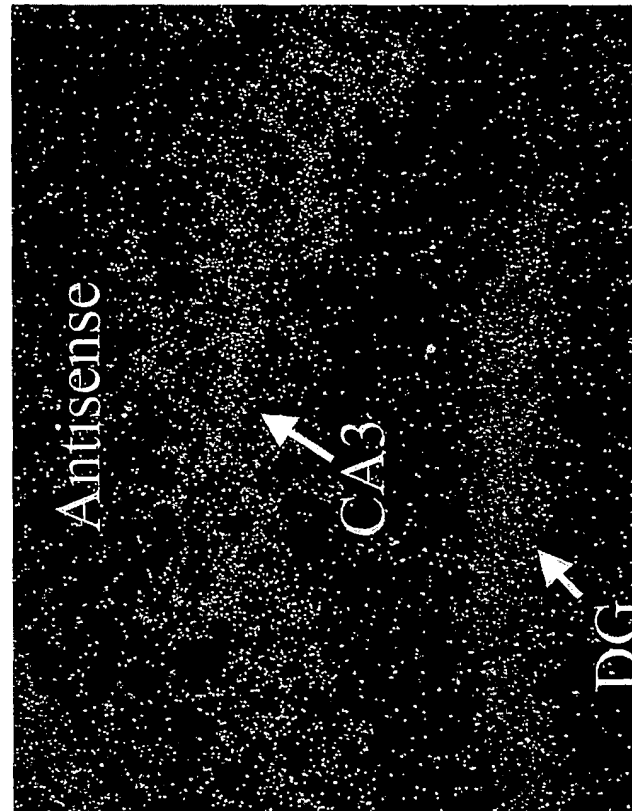


FIG. 10A



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FIG. 11

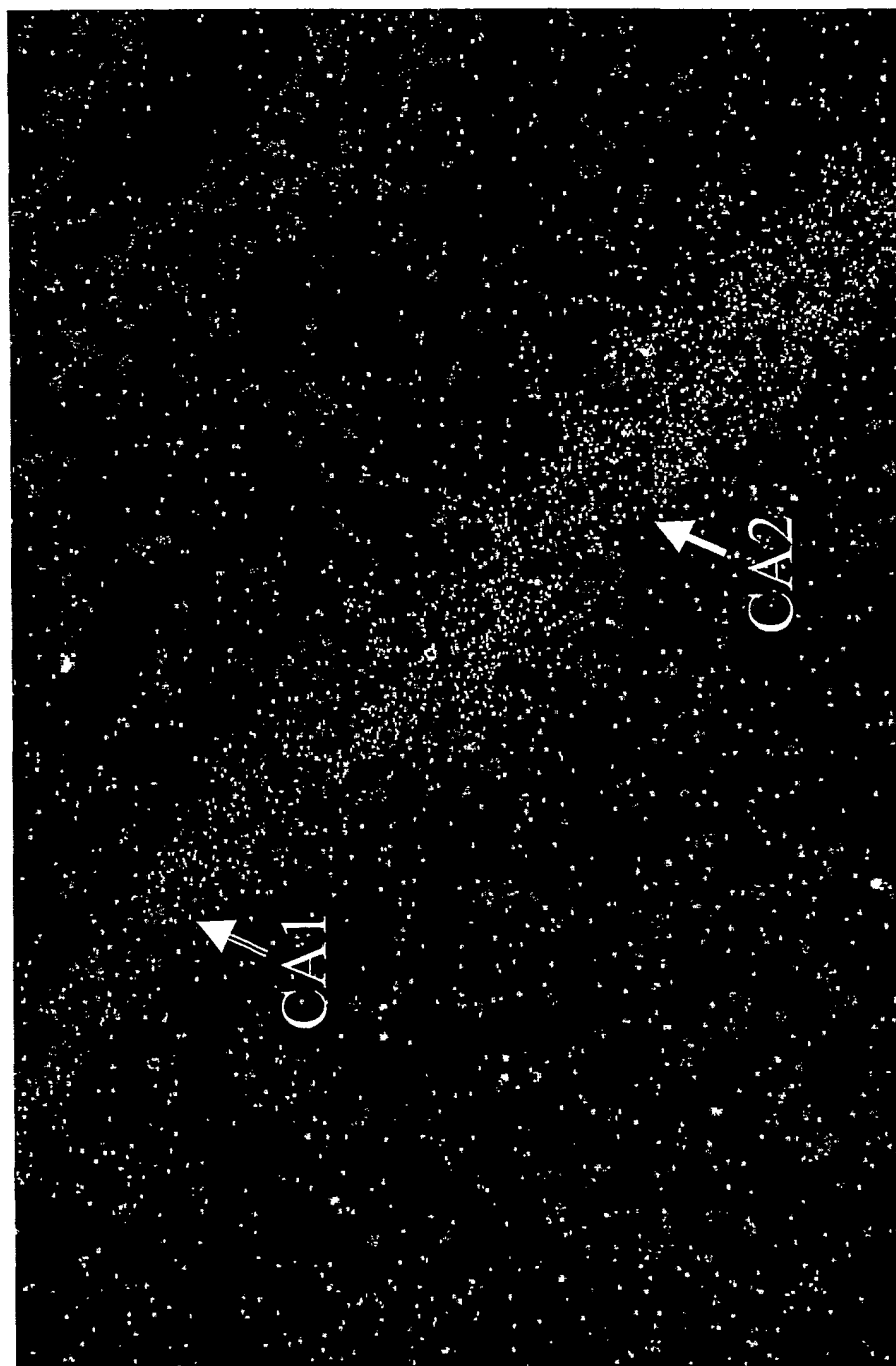


FIG. 12B

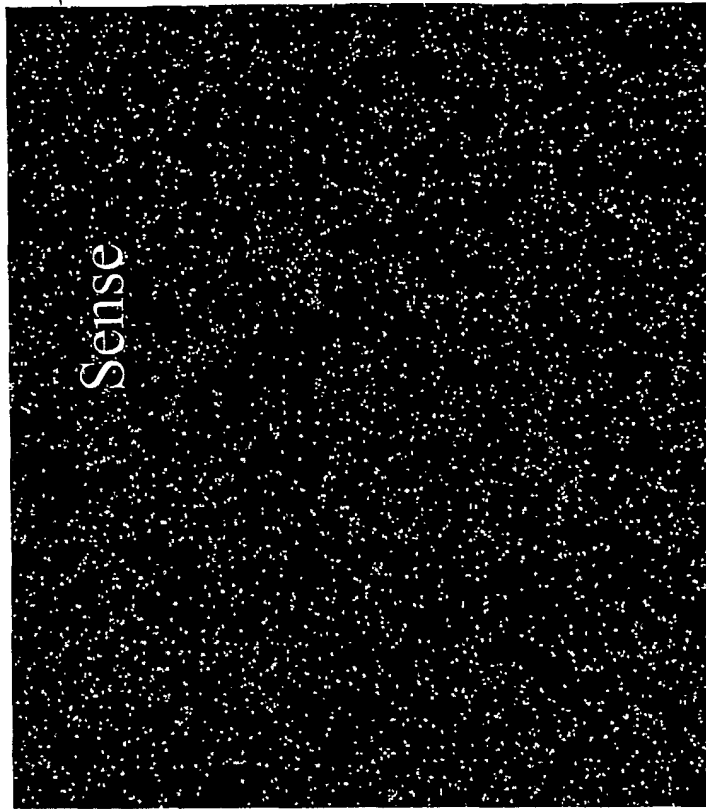


FIG. 12A

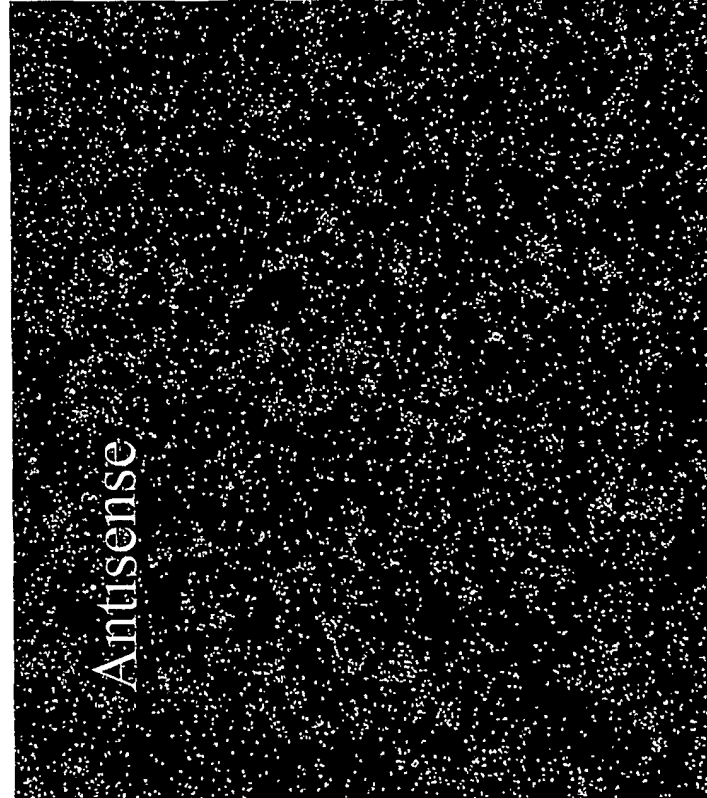


FIG. 13B

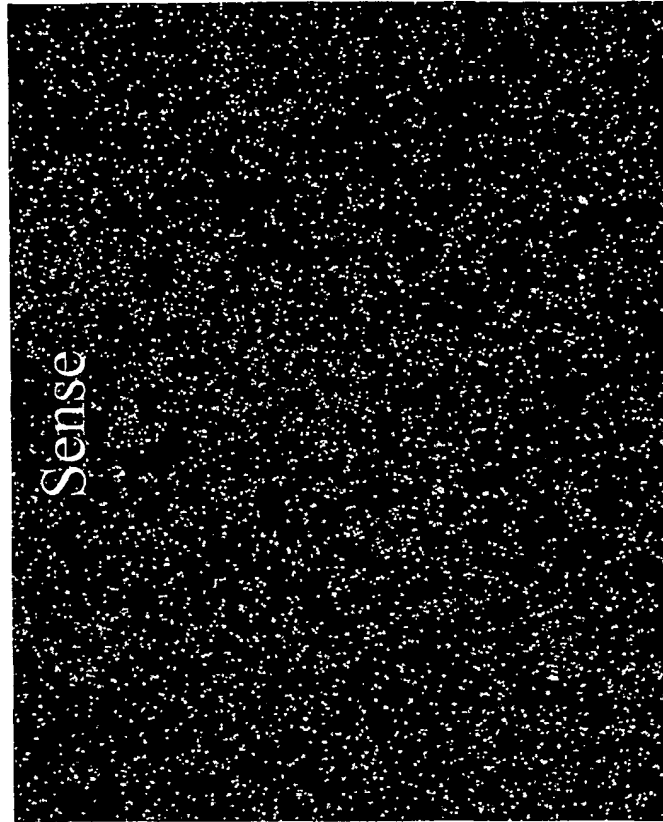


FIG. 13A

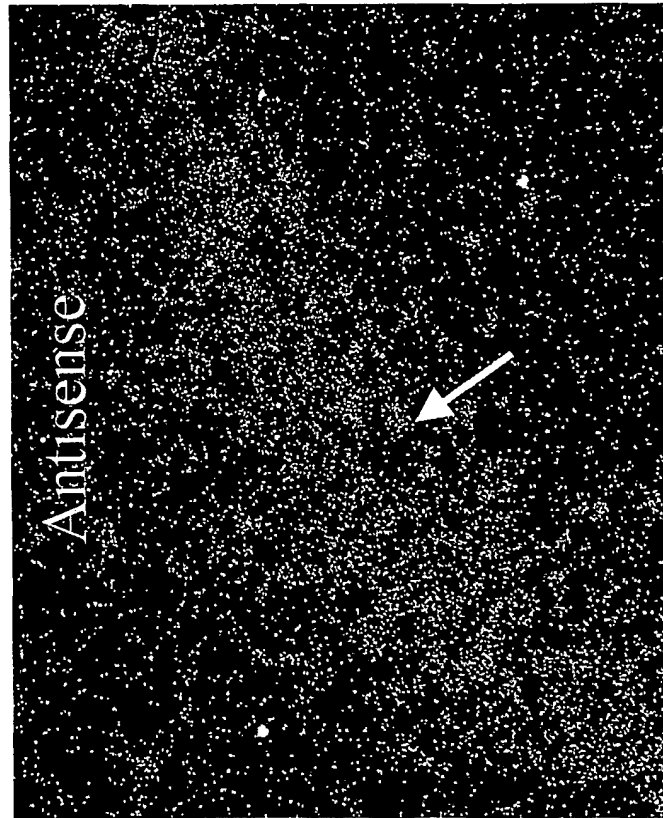


FIG. 14B

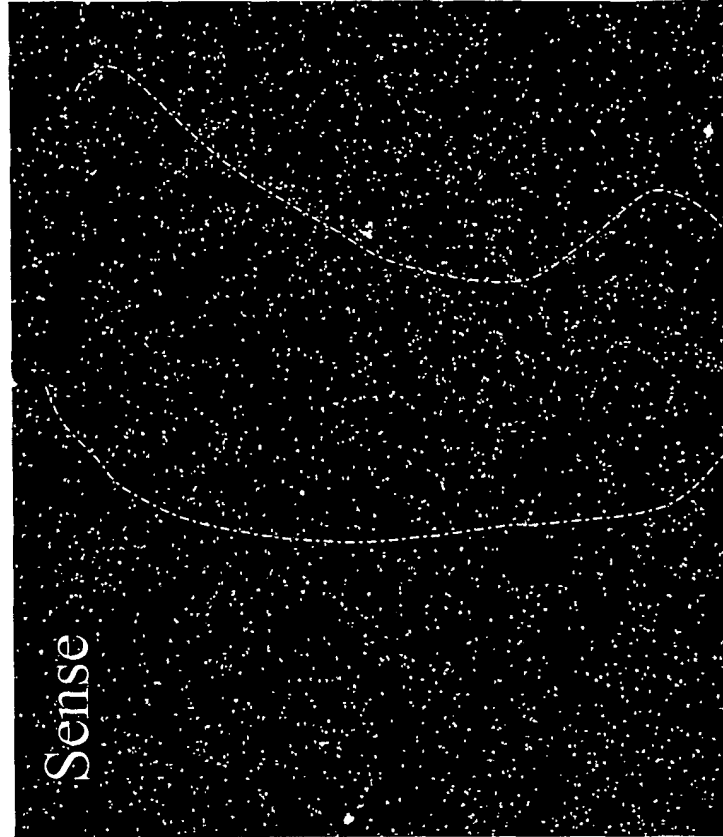
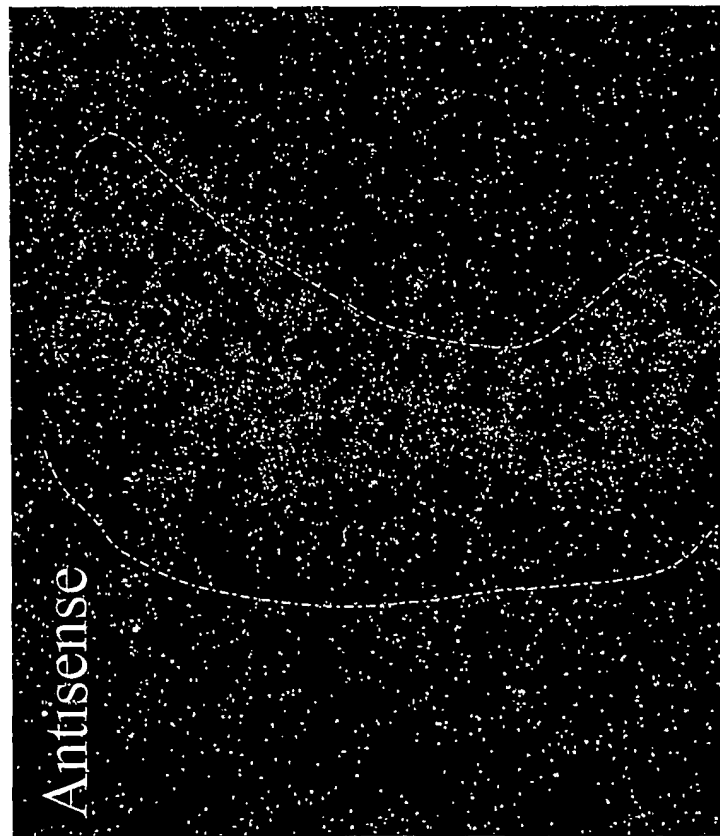


FIG. 14A



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FIG. 15B

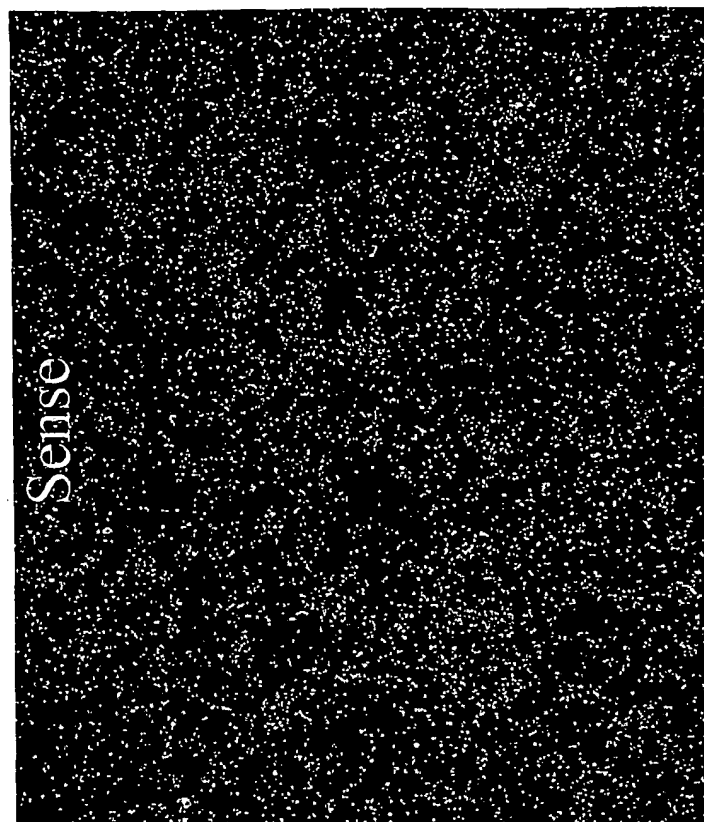


FIG. 15A

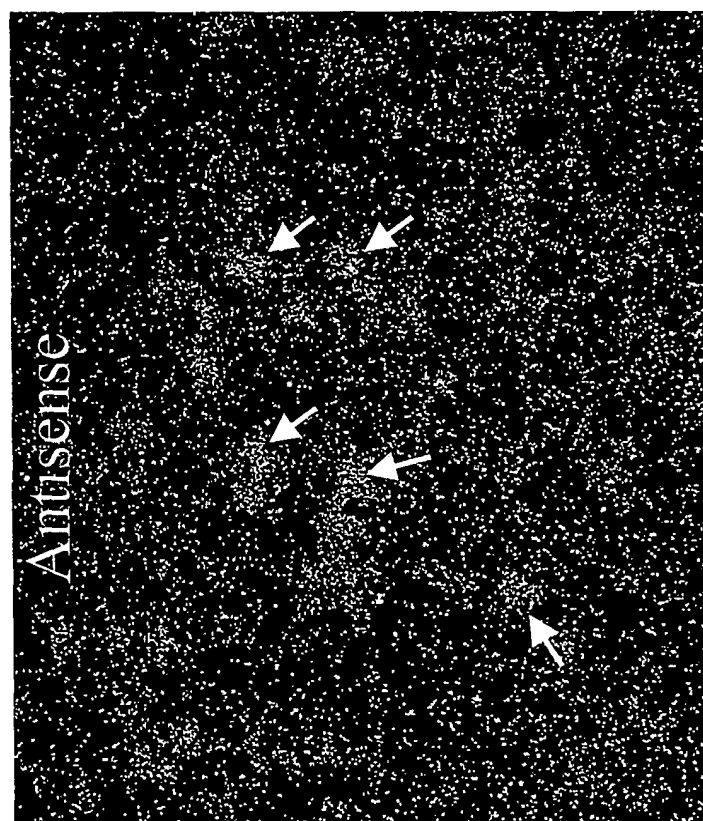


FIG. 16B

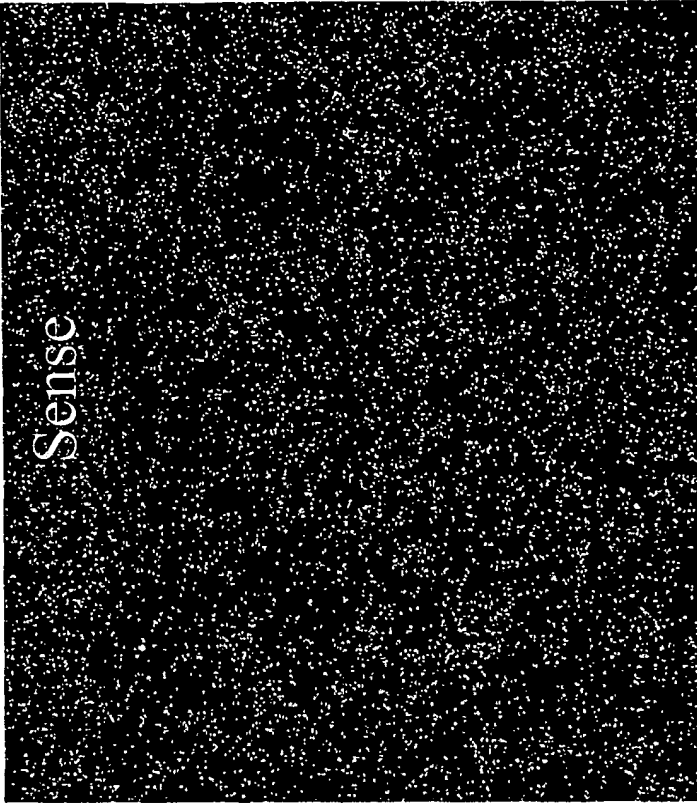


FIG. 16A

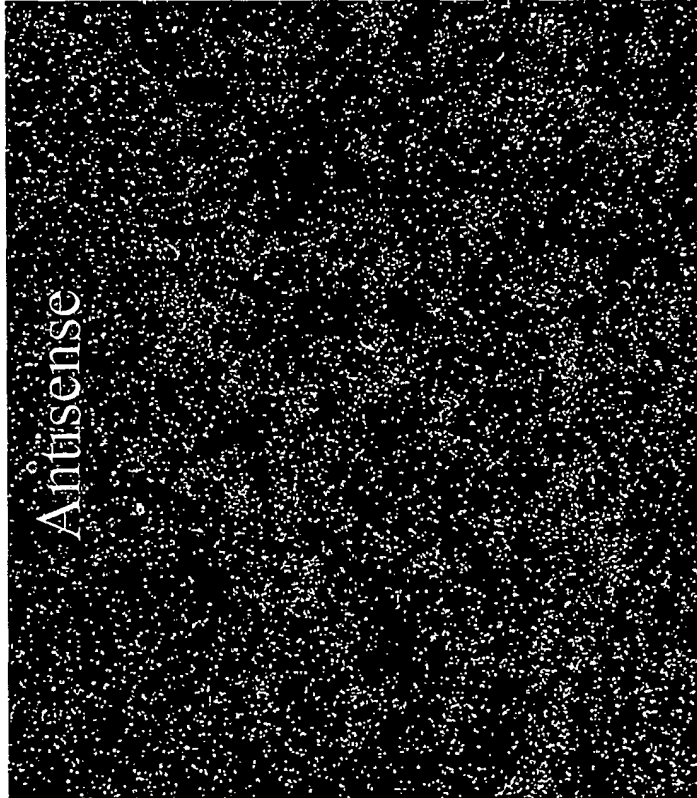


FIG. 17B

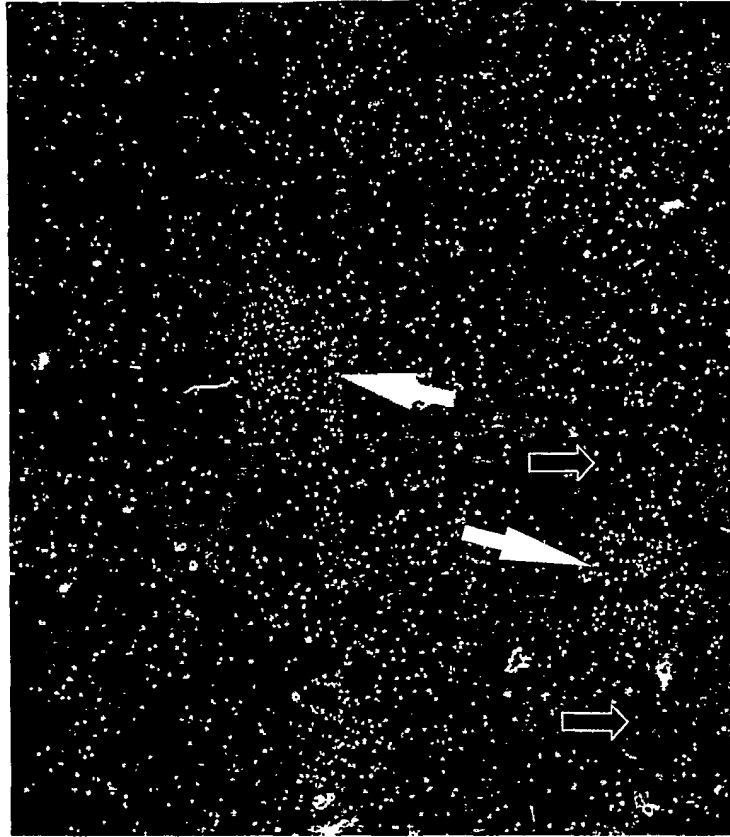
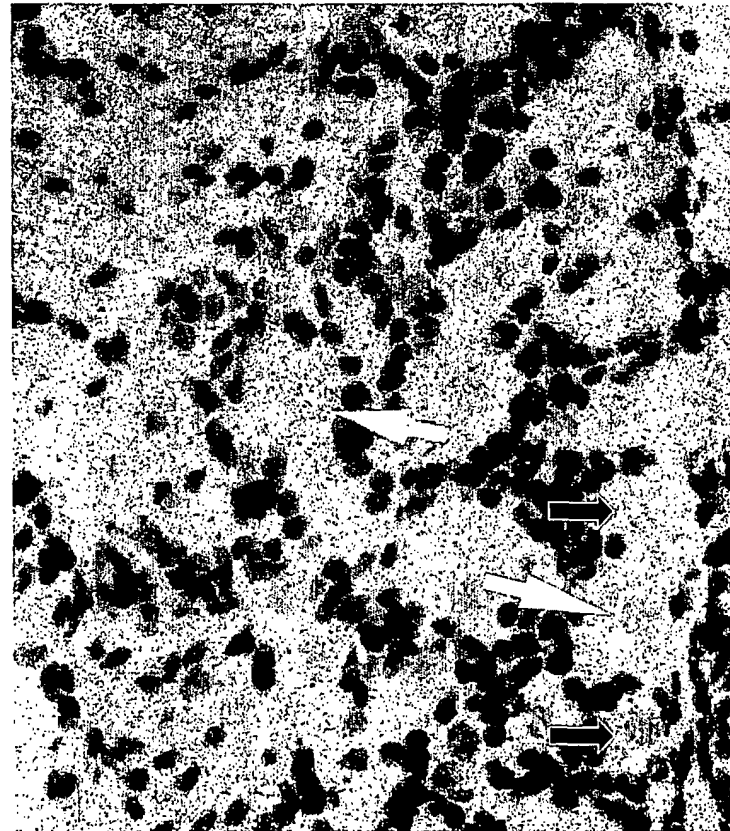


FIG. 17A



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FIG. 18B

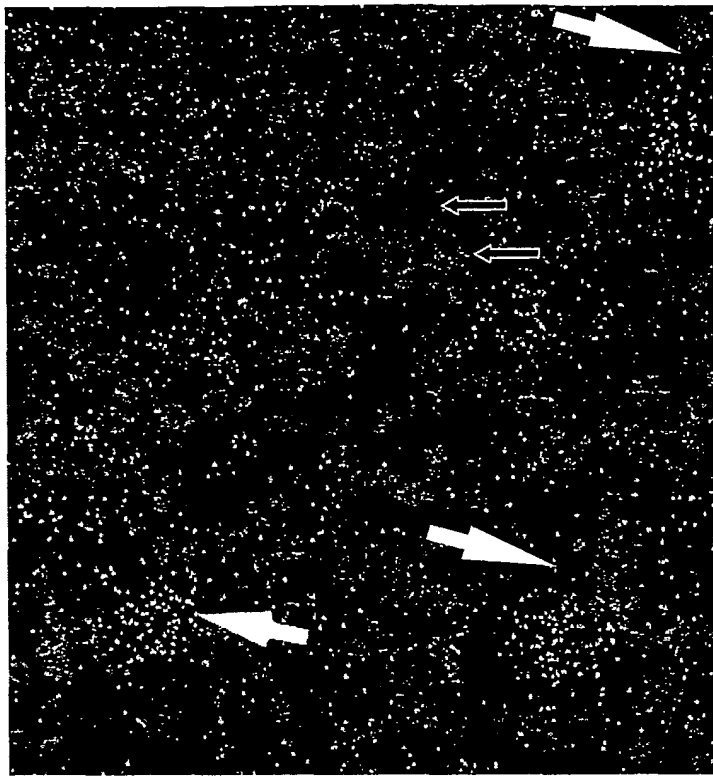
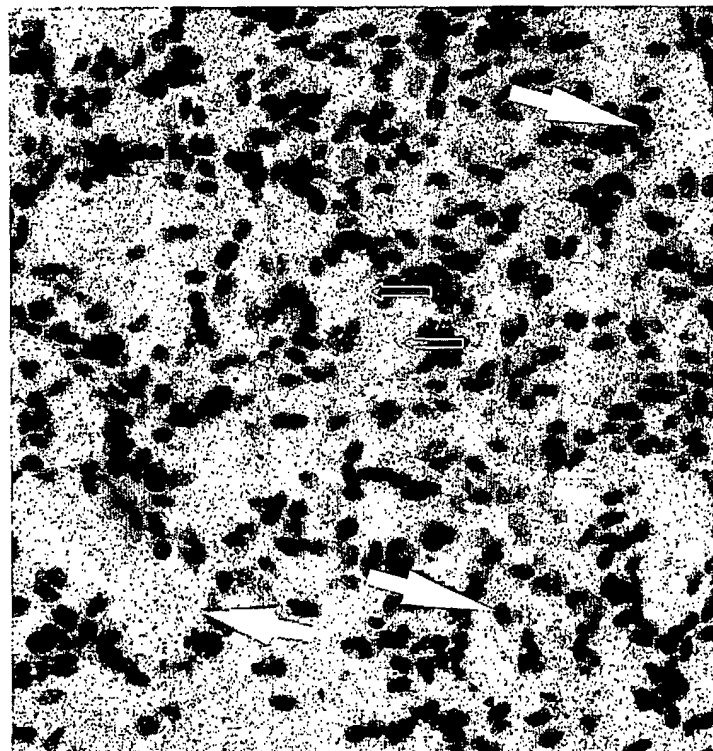


FIG. 18A



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 Arg Arg Leu Asp Gln Ser Ile Gly Lys Pro Ser Leu Phe Ile Ser Val
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 Asn Arg Val Glu Asp Lys Val Thr Gln Leu Asp Gln Arg Leu Ala Leu
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Ile Thr Asp Met Leu His Gln Leu Leu Ser Leu His Gly Gly Ser Thr
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Asp Ser Thr Arg Asp Gly Ala Leu Leu Ile Ala Gly Ser Glu Ala Pro
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Lys Arg Gly Ser Ile Leu Ser Lys Pro Arg Ala Gly Gly Ala Gly Ala
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Gly Lys Pro Pro Lys Arg Asn Ala Phe Tyr Arg Lys Leu Gln Asn Phe
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Leu Tyr Asn Val Leu Glu Arg Pro Arg Gly Trp Ala Phe Ile Tyr His
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Ser Thr Ile Lys Glu Tyr Glu Lys Ser Ser Glu Gly Ala Leu Tyr Ile
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Arg Arg Leu Gln Asn Trp Val Tyr Asn Val Leu Glu Arg Pro Arg Gly
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Ile Asp Phe Ile Val Phe Val Ala Ser Val Ala Val Ile Ala Ala Gly
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Tyr Asp Ser Ile Leu Pro Ser Phe Arg Glu Leu Ala Leu Leu Phe Glu
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Ser Val Ser Thr Asn Met Asp
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/17314

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/705 C07K16/28 C07K17/00 C12N5/10
 C12Q1/68 A61K38/00 A61K31/7088 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

SEQUENCE SEARCH, EPO-Internal, EMBL, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>LERCHE C ET AL: "Molecular cloning and functional expression of KCNQ5, a potassium channel subunit that may contribute to neuronal M-current diversity"</p> <p>JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US,</p> <p>vol. 275, no. 29,</p> <p>21 July 2000 (2000-07-21), pages 22395-22400, XP002169157</p> <p>ISSN: 0021-9258</p> <p>On-line version appeared 27.04.2000</p> <p>the whole document</p> <p style="text-align: center;">--- -/--</p>	1-27



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

* & * document member of the same patent family

Date of the actual completion of the international search

25 October 2001

Date of mailing of the international search report

08/11/2001

Name and mailing address of the ISA

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 NL - 2280 HV Rijswijk
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 Fax: (+31-70) 340-3016

Authorized officer

Aslund, J

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/17314

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 00 77035 A (NEUROSEARCH AS) 21 December 2000 (2000-12-21) the whole document ----	1-31, 36-42, 51-78
X,P	WO 00 61606 A (METZKER MICHAEL L ;CASKEY C THOMAS (US); LI WEN (US); MERCK & CO I) 19 October 2000 (2000-10-19) the whole document ----	1-31, 36-42, 51-78
X	DATABASE EBI 'Online! Hinxton, UK; Accession number: AF202977, 14 May 2000 (2000-05-14) XP002180460 Nucleotides 1-2694 ----	1-27
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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 32-35, 43-50

Present claims 32-35, 43-50 relate to compounds defined by reference to a desirable characteristic or property, namely the ability to modulate the activity of KCNQ5.

The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, no the search has been carried out for claims 32-35 and 43-50.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.